# 7. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

## 7.1. INTRODUCTION

The particulate phase of diesel exhaust is composed of aggregates of carbon particles; the primary particle diameter ranges from 10 to 80 nm, and aggregates of these primary particles have mass median diameters averaging 0.2 to 0.3 μm (Vuk et al., 1976; Carpenter and Johnson, 1979), although some may approach 1.0 μm. A variety of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), are adsorbed to this carbon core (see Tables 2-8 and 2-10) and comprise 5% to 65% of the total particle mass (Cuddihy et al., 1984). Some of these organic compounds, such as benzo[a]pyrene (B[a]P), dinitropyrenes, and 1-nitropyrene, have received special attention regarding their carcinogenic and mutagenic potential. These organics may be strongly or weakly bound to the carbon core and represent varying amounts of the total particle mass. Qualitative and quantitative relationships for these organics depend on such variables as fuel composition, engine design, and engine operating conditions. Although less emphasis has been placed on the gaseous phase, potential carcinogens such as formaldehyde, acetaldehyde, benzene, as well as lower molecular weight PAHs, may also be present in this fraction.

The respirability of these particles and their associated organics provides a basis for health hazard concerns, and the reported mutagenicity (Huisingh et al., 1978) and skin papilloma induction (Kotin et al., 1955) of solvent extracts of diesel particulate matter (DPM) suggests a potential for carcinogenicity. Zamora et al. (1983) provided evidence that DPM extracts contained components that acted as weak tumor promoters in vitro. Recently, emphasis has been directed toward assessing the carcinogenic potential of whole and filtered diesel exhaust using whole-animal studies and understanding the mechanisms and implications of deposition, retention, and clearance of the particulate phase of diesel exhaust.

This chapter summarizes studies that assess the carcinogenic potential of diesel exhaust in laboratory animals. Experimental protocols for the inhalation studies usually consisted of exposure (usually chronic) to diluted exhaust in whole-body exposure chambers using rats, mice, and hamsters as model species. Some of these studies used both filtered (free of particulate matter) diesel exhaust and unfiltered (whole) diesel exhaust to differentiate gaseous-phase effects from effects induced by DPM and its adsorbed components. Inhalation exposure to DPM alone, however, was not reported. Particulate matter concentrations in the diesel exhaust used in these studies ranged from 0.1 to 12 $mg/m^3$. Clean air (usually filtered) was used in the control exposures. Studies providing both positive, negative, or inconclusive findings have been reported. In this chapter, any indication of statistical significance implies that $p \leq 0.05$ was reported in the reviewed publications. The experimental protocols and exposure atmosphere characterizations

are not described in detail here but may be found in Appendix A. A summary of the animal carcinogenicity studies and their results is presented in Table 7-1.

Also included are studies that assessed the carcinogenic and tumorigenic effects of DPM and solvent extracts of these particles following dermal application, subcutaneous (s.c.) injection, intraperitoneal (i.p.) injection, or intratracheal (itr.) instillation in rodents, as well as co-carcinogenicity studies. Individual chemicals present in the gaseous phase or adsorbed to the particle surface were not included in this review because adequate assessments of those of likely concern (i.e., formaldehyde, acetaldehyde, benzene, PAHs) have been published in other health assessment documents.

## 7.2. INHALATION STUDIES

### 7.2.1. Rat Studies

Mauderly et al. (1987) provided data affirming the carcinogenicity of automotive diesel engine exhaust in F344/Crl rats following chronic inhalation exposure. Male and female rats were exposed to diesel engine exhaust at nominal DPM concentrations of 0.35 (n = 366), 3.5 (n = 367), or 7.1 (n = 364) mg/m$^3$ for 7 h/day, 5 days/week for up to 30 mo. Sham-exposed (n = 365) controls breathed filtered room air. A total of 230, 223, 221, and 227 of these rats (sham-exposed, low-, medium-, and high-exposure groups, respectively) were examined for lung tumors. These numbers include those animals that died or were euthanized during exposure and those that were terminated following 30 mo of exposure. The exhaust was generated by 1980 model 5.7-L Oldsmobile V-8 engines operated through continuously repeating U.S. Federal Test Procedure (FTP) urban certification cycles. The engines were equipped with automatic transmissions connected to eddy-current dynamometers and flywheels simulating resistive and inertial loads of a midsize passenger car. The D-2 diesel control fuel (Phillips Chemical Co.) met U.S. EPA certification standards and contained approximately 30% aromatic hydrocarbons and 0.3% sulfur. Following passage through a standard automotive muffler and tail pipe, the exhaust was diluted 10:1 with filtered air in a dilution tunnel and serially diluted to the final concentrations. The primary dilution process was such that particle coagulation was retarded. Mokler et al. (1984) provided a detailed description of the exposure system. The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A. No exposure-related changes in body weight or life span were noted for any of the exposed animals nor were there any signs of overt toxicity. Collective lung tumor incidence was greater (z statistic, $p \leq 0.05$) in the high (7.1 mg/m$^3$) and medium (3.5 mg/m$^3$) exposure groups (12.8% and 3.6%, respectively) versus the control and low (0.35 mg/m$^3$) exposure groups (0.9% and 1.3%,

## Table 7-1. Summary of animal carcinogenicity studies

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Adenocarcinoma + squamous cell carcinomas | Squamous cysts | All tumors | |
| Mauderly et al. (1987) | Rat/F344 | M + F, 230[b] | Clean air | 0 | None | 7 h/day, | NA | (0) | (0.9) | (0) | (0.9) | |
| | | M + F, 223 | Whole exhaust | 0.35 | None | 5 days/week | | (0) | (1.3) | (0) | (1.3) | |
| | | M + F, 221 | Whole exhaust | 3.5 | None | up to 30 mo | | (2.3) | (0.5) | (0.9) | (3.6)[c] | |
| | | M + F, 227 | Whole exhaust | 7.1 | None | | | (0.4) | (7.5) | (4.9) | (12.8)[c] | |
| | | | | | | | | Adenomas | Adenocarcinomas | Squamous cell carcinoma | Adeno-squamous carcinoma | Other neoplasms |
| Nikula et al. (1995) | Rat/F344 | M + F, 214[b] | Clean air | 0 | None | 16 h/day, | 6 weeks | 1/214 (<1) | 1/214 (<1) | 1/214 (<1) | 0/214 (0) | 0/214 (0) |
| | | M + F, 210 | Whole exhaust | 2.5 | None | 5 days/week | | 7/210 (3) | 4/210 (2) | 3/210 (1) | 0/210 (0) | 0/210 (0) |
| | | M + F, 212 | Whole exhaust | 6.5 | None | for up to | | 23/212 (11) | 22/212 (10) | 3/212 (1) | 1/212 (<1) | 0/212 (0) |
| | | M + F, 213 | Carbon black | 2.5 | None | 24 mo | | 3/213 (1) | 7/213 (3) | 0/213 (0) | 0/213 (0) | 1/213 (<1) |
| | | M + F, 211 | Carbon black | 6.5 | None | | | 13/211 (6) | 21/211 (10) | 3/211 (1) | 2/211 (<1) | 0/211 (0) |
| | | | | | | | | Adenomas | Carcinomas | Squamous cell tumors | All tumors | |
| Heinrich et al. (1986a,b) | Rat/ Wistar | F, 96 | Clean air | 0 | None | 19 h/day, | NA | 0/96 (0) | 0/96 (0) | 0/96 (0) | 0/96 (0) | |
| | | F, 92 | Filtered exhaust | 0 | None | 5 days/week | | 0/92 (0) | 0/92 (0) | 0/92 (0) | 0/92 (0) | |
| Mohr et al. (1986) | | F, 95 | Whole exhaust | 4.0 | None | for up to 35 mo | | 8/95 (8.4) | 0/95 (0) | 9/95 (9.4) | 17/95 (17.8)[c] | |
| | | | | | | | | Adenomas | Adenocarcinoma | Squamous cell tumors | All tumors | |
| Heinrich et al. (1986a,b) | Mouse/ NMRI | M + F, 84 | Clean air | 0 | None | 19 h/day, | NA | 9/84 (11) | 2/84 (2) | — | 11/84 (13) | |
| | | M + F, 93 | Filtered exhaust | 0 | None | 5 days/week | | 11/93 (12) | 18/93 (19)[c] | — | 29/93 (31)[c] | |
| | | M + F, 76 | Whole exhaust | 4.0 | None | for up to 30 mo | | 11/76 (15) | 13/76 (17)[c] | — | 24/76 (32)[c] | |
| | Hamsters/ Syrian | M + F, 96 | Clean air | 0 | None | 19 h/day, | NA | 0/96 (0) | 0/96 (0) | 0/96 | 0/96 (0) | |
| | | M + F, 96 | Filtered exhaust | 0 | None | 5 days/week | | 0/96 (0) | 0/96 (0) | 0/96 | 0/96 (0) | |
| | | M + F, 96 | Whole exhaust | 4.0 | None | for up to 30 mo | | 0/96 (0) | 0/96 (0) | 0/96 | 0/96 (0) | |

## Table 7-1. Summary of animal carcinogenicity studies (continued)

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Squamous cell carcinoma | All lung tumors | |
| Henrich et al. (1989a) | Rat/ Wistar | F, NS | Clean air | 0 | DPN[d] | 19 h/day, 5 days/week for 24 to 30 mo | NA | | | (4.4) | (84.8) | |
| | | F, NS | Whole exhaust | 4.2 | DPN[d] | | | | | (46.8)[c] | (83.0) | |
| | | F, NS | Filtered exhaust | 0 | DPN[d] | | | | | (4.4) | (67.4) | |
| | | F, NS | Clean air | 0 | DPN[e] | | | | | (16.7) | (93.8) | |
| | | F, NS | Whole exhaust | 4.2 | DPN[e] | | | | | (31.3)[c] | (89.6) | |
| | | F, NS | Filtered exhaust | 0 | DPN[e] | | | | | (14.6) | (89.6) | |
| | | | | | | | | Adenomas | Adenocarcinomas | Squamous cell carcinomas | Benign squamous cell tumors | |
| Heinrich et al. (1995) | Rat/ Wistar | F, 220 | Clean air | 0 | None | 18 h/day, 5 days/week, for up to 24 mo | 6 mo | 0/217 (0) | 1/217 (<1) | 0/217 (0) | 0/217 (0) | |
| | | F, 200 | Whole exhaust | 0.8 | None | | | 0/198 (0) | 0/198 (0) | 0/198 (0) | 0/198 (0) | |
| | | F, 200 | Whole exhaust | 2.5 | None | | | 2/200 (1) | 1/200 (<1) | 0/200 (0) | 7/200 (3.5) | |
| | | F, 100 | Whole exhaust | 7.0 | None | | | 4/100 (4) | 4/100 (4) | 2/100 (2) | 14/100 (14) | Tumor incidences after 30 mo |
| | | F, 100 | Carbon black | 11.6 | None | | | 13/100 (13) | 13/100 (13) | 4/100 (4) | 20/100 (20) | |
| | | F, 100 | $TiO_2$ | 10.0 | None | | | 4/100 (4) | 13/100 (13) | 3/100 (3) | 20/100 (20) | |
| | Mouse/ C57BL/ 6N | F, 120 | Clean air | 0 | None | 18 h/day, 5 days/week, for up to 21 mo | 6 mo | | | | | 5.1% tumor rate |
| | | F, 120 | Whole exhaust | 4.5 | None | | | | | | | 8.5% tumor rate |
| | | F, 120 | Particle-free exhaust | 0 | None | | | | | | | 3.5% tumor rate |
| | Mouse/ NMRI | F, 120 | Clean air | 0 | None | 18 h/day, 5 days/week for up to 13.5 mo | 9.5 mo | (25) | (15.4) | | | |
| | | F, 120 | Whole exhaust | 4.5 | None | | | (21.8) | (15.4) | | | |
| | | | Carbon black | 11.6 | None | | | (11.3) | (10) | | | |
| | | | $TiO_2$ | 10 | None | | | (11.3) | (2.5) | | | |
| | Mouse/ NMRI | F,120 | Clean air | 0 | None | 18 h/day, 5 days/week, 23 mo | None | (25) | (8.8) | | | |
| | | F,120 | Whole exhaust | 4.5 | None | | | (18.3) | (5.0) | | | |
| | | F,120 | Particle-free exhaust | 0 | None | | | (31.7) | (15) | | | |

## Table 7-1. Summary of animal carcinogenicity studies (continued)

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Adenosquamous carcinomas | Squamous cell carcinomas | All tumors | |
| Takaki et al. (1989) Light-duty engine | Rat/F344 | M + F, 123 | Clean air | 0 | None | 16 h/day, 6 days/week, for up to 30 mo | NA | 1/23 (0.8) | 2/123 (1.6) | 1/23 (0.8) | 4/123 (3.3) | |
| | | M + F, 123 | Whole exhaust | 0.1 | None | | | 1/23 (0.8) | 1/23 (0.8) | 1/23 (0.8) | 3/123 (2.4) | |
| | | M + F, 125 | Whole exhaust | 0.4 | None | | | 1/25 (0.8) | 0/125 (0) | 0/125 (0) | 1/125 (0.8) | |
| | | M + F, 123 | Whole exhaust | 1.1 | None | | | 0/23 (0) | 5/123 (4.1) | 0/123 (0) | 5/123 (4.1) | |
| | | M + F, 124 | Whole exhaust | 2.3 | None | | | 1/24 (8.1) | 2/124 (1.6) | 0/124 (0) | 3/124 (2.4) | |
| | | | | | | | | Adenomas | Adenosquamous carcinomas | Squamous cell carcinomas | All tumors | |
| Ishinishi et al. (1988a) Heavy-duty engine | Rat/F344 | M + F, 123 | Clean air | 0 | None | 16 h/day, 6 days/week, for up to 30 mo | NA | 0/123 (0) | 1/123 (0.8) | 0/123 (0) | 1/123 (0.8) | |
| | | M + F, 123 | Whole exhaust | 0.5 | None | | | 0/123 (0) | 0/123 (0) | 1/123 (0.8) | 1/123 (0.8) | |
| | | M + F, 125 | Whole exhaust | 1.0 | None | | | 0/125 (0) | 0/125 (0) | 0/125 (0) | 0/125 (0) | |
| | | M + F, 123 | Whole exhaust | 1.8 | None | | | 0/123 (0) | 4/123 (3.3) | 0/123 (0) | 4/123 (3.3) | |
| | | M + F, 124 | Whole exhaust | 3.7 | None | | | 0/124 (0) | 6/124 (4.8) | 2/124 (1.6) | 8/124 (6.5)[c] | |
| | | | | | | | | Adenomas | Adenocarcinoma and adenosquamous carcinoma | Large cell and squamous cell carcinomas | All tumors | |
| Iwai et al. (1986) | Rat/F344 | F, 24 | Clean air | 0 | None | 8 h/day, 7 days/week, for 24 mo | NA | 1/22 (4.5) | 0/22 (0) | 0/22 (0) | 1/22 (4.5)[f] | |
| | | F, 24 | Filtered exhaust | 0 | None | | | 0/16 (0) | 0/16 (0) | 0/16 (0) | 0/16 (0) | |
| | | F, 24 | Whole exhaust | 4.9 | None | | | 3/19 (0) | 3/19 (15.8) | 2/19 (10.5) | 8/19 (42.1)[c,g] | |
| | | | | | | | | | Adenoma | Carcinoma | | |
| Takemoto et al. (1986) | Rat/F344 | F, 12 | Clean air | 0 | None | 4 h/day, 4 days/week, 18-24 mo | NA | | 0/12 (0) | 0/12 (0) | | |
| | | F, 21 | Clean air | 0 | DIPN[h] | | | | 10/21 (47.6) | 4/21 (19) | | |
| | | F, 15 | Whole exhaust | 2-4 | None | | | | 0/15 (0) | 0/15 (0) | | |
| | | F, 18 | Whole exhaust | 2-4 | DIPN[h] | | | | 12/18 (66.7) | 7/18 (38.9) | | |
| | | | | | | | | | Adenoma | Adeno-carcinoma | | |
| | Mouse/ IRC | M + F, 45 | Clean air | 0 | None | 4 h/day, 4 days/week, for 19-28 mo | NA | | 3/45 (6.7) | 1/45 (2.2) | | |
| | | M + F, 69 | Whole exhaust | 2-4 | None | | | | 6/69 (8.7) | 3/69 (4.3) | | |
| | Mouse/ C57BL | M + F, 12 | Clean air | 0 | None | 4 h/day, 4 days/week for 19-28 mo | NA | | 1/12 (8.3) | 0/12 (0) | | |
| | | M + F, 38 | Whole exhaust | 2-4 | None | | | | 8/38 (21.1) | 3/38 (7.9) | | |

## Table 7-1. Summary of animal carcinogenicity studies (continued)

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Primary lung tumors | |
| Brightwell et al. (1989) | Rat/344 | M + F, 260 | Clean air | 0 | None | 16 h/day, 5 days/week, for 24 mo | NA | 3/260 (1.2) | Tumor incidence for all rats dying or sacrificed |
| | | M + F, 144 | Filtered exhaust (medium exposure) | 0 | None | | | 0/144 (0) | |
| | | M + F, 143 | Filtered exhaust (high exposure) | 0 | None | | | 0/143 (0) | |
| | | M + F, 143 | Whole exhaust | 0.7 | None | | | 1/143 (0.7) | ♀ 24/25 (96%) after 24 mo ♂ 12/27 (44%) after 24 mo |
| | | M + F, 144 | Whole exhaust | 2.2 | None | | | 14/144 (9.7)[c] | |
| | | M + F, 143 | Whole exhaust | 6.6 | None | | | 55/143 (38.5)[c] | |
| | | | | | | | | Primary lung tumors | |
| | Hamster/ Syrian Golden | M + F, | Clean air | 0 | None | 16 h/day, 5 days/week, for 24 mo | NA | 7/202 (3.5) | Respiratory tract tumors not related to exhaust exposure for any of the groups |
| | | M + F, 202 | Clean air | 0 | DEN[j] | | | 4/104 (3.8) | |
| | | M + F, 104 | Filtered exhaust (medium dose) | 0 | DEN[j] | | | 9/104 (8.7) | |
| | | M + F, 104 | Filtered exhaust (high dose) | 0 | DEN[j] | | | 2/101 (2.0) | |
| | | M + F, 101 | Whole exhaust | 0.7 | DEN[j] | | | 6/102 (5.9) | |
| | | M + F, 102 | Whole exhaust | 2.2 | DEN[j] | | | 4/101 (3.9) | |
| | | M + F, 101 | Whole exhaust | 6.6 | DEN[j] | | | 1/204 (0.5) | |
| | | M + F, 204 | Filtered exhaust (high dose) | 0 | None | | | 0/203 (0) | |
| | | M + F, 203 | Whole exhaust | 6.6 | None | | | | |
| | | | | | | | | Adenomas | |
| Karagianes et al. (1981) | Rat/ Wistar | M, 40 | Clean air | 0 | None | 6 h/day, 5 days/week, for up to 20 mo | NA | 0/6 (0) | |
| | | M, 40 | Whole exhaust | 8.3 | None | | | 1/6 (16.6) | |

## Table 7-1. Summary of animal carcinogenicity studies (continued)

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Lung Tumors | |
| Orthoefer et al. (1981) (Pepelko and Peirano, 1983) | Mouse/ Strong A | M, 25 | Clean air | 0 | None | 20 h/day, 7 days/week, for 7 weeks | | 3/22 (13.6) | 0.13 Tumors/ mouse |
| | | | Whole exhaust | 6.4 | None | | 26 weeks | 7/19 (36.8) | 0.63 Tumors/ mouse |
| | | | Whole exhaust | 6.4 | UV irradiated | | 26 weeks | 6/22 (27.3) | 0.27 Tumors/ mouse |
| | | | | | | | | Lung Tumors | |
| | Mouse/ Jackson A | M + F, 40 | Clean air | 0 | None | 20 h/day, 7 days/week, for 8 weeks | 8 weeks | 16/36 (44.4) | 0.5 Tumors/ mouse |
| | | M + F, 40 | Whole exhaust | 6.4 | None | | 8 weeks | 11/34 (32.3) | 0.4 Tumors/ mouse |
| | Mouse/ Jackson A | F, 60 | Clean air | 0 | None | 20 h/day, 7 days/week, for approx. 7 mo. | | 4/58 (6.9) | 0.09 Tumors/ mouse |
| | | F, 60 | Clean air | 0 | Urethan[l] | | | 9/52 (17.3) | 0.25 Tumors/ mouse |
| | | F, 60 | Whole exhaust | 6.4 | None | | | 14/56 (25.0) | 0.32 Tumors/ mouse |
| | | F, 60 | Whole exhaust | 6.4 | Urethan[k] | | | 22/59 (37.3) | 0.39 Tumors/ mouse |
| | | M, 429 | Clean air | 0 | None | | | 73/403 (18.0) | 0.23 Tumors/ mouse |
| | | M, 430 | Whole exhaust | 6.4 | None | | | 66/368 (17.9) | 0.20 Tumors/ mouse |

## Table 7-1. Summary of animal carcinogenicity studies (continued)

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Carcinomas | All tumors | |
| Pepelko and Peirano (1983) | Mouse/ Sencar | M + F, 260 | Clean air | 0 | None | Continuous | NA | (5.1) | (0.5) | (5.6) | |
| | | | Clean air | 0 | BHT[l] | for 15 mo | | (12.2) | (1.7) | (2.8) | |
| | | | Clean air | 0 | Urethan[k] | | | (8.1) | (0.9) | (9.0) | |
| | | | Whole exhaust | 12 | None | | | (10.2)[c] | (1.0) | (11.2)[c] | |
| | | | Whole exhaust | 12 | BHT[l] | | | (5.4) | (2.7) | (8.1) | |
| | | | Whole exhaust | 12 | Urethan[l] | | | (8.7) | (2.6) | (11.2) | |
| | | | | | | | | | All tumors | | |
| | Mouse/ Strain A | M + F, 90 | Clean air | 0 | None | | NA | | 21/87 (24) | | 0.29 Tumors/ mouse |
| | | | Clean air | 0 | Exposure (darkness) | | | | 59/237 (24.9) | | 0.27 Tumors/ mouse |
| | | | Whole exhaust | 12 | Exposure | | | | 10/80 12.5) | | 0.14 |
| | | | Whole exhaust | 12 | (darkness) | | | | 22/250 (0.10) | | 0.10 |
| | | | Clean air | 0 | Urethan[m] | | | | 66/75 (88) | | 2.80 |
| | | | Whole exhaust | 12 | Urethan[m] | | | | 42/75 (0.95) | | 0.95 |
| | | | | | | | | | Broncho-alveolar carcinoma | | |
| Kaplan et al. (1983) White et al. (1983) | Rat/F344 | M, 30 | Clean air | 0 | None | 20 h/day, | 8 mo | | 0/30 (0) | | |
| | | M, 30 | Whole exhaust | 0.25 | None | 7 days/week, | 8 mo | | 1/30 (3.3) | | |
| | | M, 30 | Whole exhaust | 0.75 | None | for up to | 8 mo | | 3/30 (10.0) | | |
| | | M, 30 | Whole exhaust | 1.5 | None | 15 mo | 8 mo | | 1/30 (3.3) | | |
| | | | | | | | | | Pulmonary adenoma | | |
| | Mouse/ A/J | M, 388 | Clean air | 0 | None | 20 h/day, | NA | | 130/388 (33.5) | | |
| | | M, 388 | Whole exhaust | 0.25 | None | 7 days/week, | | | 131/388 (33.8) | | |
| | | M, 399 | Whole exhaust | 0.75 | None | for up to | | | 109/399 (27.3) | | |
| | | M, 396 | Whole exhaust | 1.5 | None | 8 mo | | | 99/396 (25.0) | | |
| | | | | | | | | | Pulmonary adenomas | | |
| Kaplan et al. (1982) | Mouse/A/ J | M, 458 | Clean air | 0 | None | 20 h/day, | 6 mo | | 144/458 (31.4) | | |
| | | M, 18 | Clean air | 0 | Urethan[k] | 7 days/week, | | | 18/18 (100) | | |
| | | M, 485 | Whole exhaust | 1.5 | None | for 3 mo | | | 165/485 (34.2) | | |

## Table 7-1. Summary of animal carcinogenicity studies (continued)

| Study | Species/ strain | Sex/total number | Exposure atmosphere | Particle concentration ($mg/m^3$) | Other treatment | Exposure protocol | Postexposure observation | Tumor type and incidence (%) [a] | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Carcinomas | All tumors | | | |
| Ishinishi et al. (1988a) | Rat/F344 | NS, 5 | Whole exhaust | 0.1 | None | 16 h/day, | 6 mo | 0/5 (0) | 0/5 (0) | 0/5 (0) | | | |
| | | NS, 8 | Whole exhaust | 0.1 | None | 6 days/week, | 12 mo | 0/8 (0) | 0/8 (0) | 0/8 (0) | | | |
| | | NS, 11 | Whole exhaust | 0.1 | None | for 12 mo | 18 mo | 0/11 (0) | 0/11 (0) | 0/11 (0) | | | |
| Light duty | | NS, 5 | Whole exhaust | 1.1 | None | | 6 mo | 0/5 (0) | 0/5 (0) | 0/5 (0) | | | |
| | | NS, 9 | Whole exhaust | 1.1 | None | | 12 mo | 0/9 (0) | 0/9 (0) | 0/9 (0) | | | |
| | | NS, 11 | Whole exhaust | 1.1 | None | | 18 mo | 0/11 (0) | 0/11 (0) | 0/11 (0) | | | |
| Heavy duty | | NS, 5 | Whole exhaust | 0.5 | None | 16 h/day, | 6 mo | 0/5 (0) | 0/5 (0) | 0/5 (0) | | | |
| | | NS, 9 | Whole exhaust | 0.5 | None | 6 days/week, | 12 mo | 0/9 (0) | 0/9 (0) | 0/9 (0) | | | |
| | | NS 11 | Whole exhaust | 0.5 | None | for 12 mo | 18 mo | 0/11 (0) | 0/11 (0) | 0/11 (0) | | | |
| | | NS, 5 | Whole exhaust | 1.8 | None | | 6 mo | 0/5 (0) | 0/5 (0) | 0/11 (0) | | | |
| | | NS, 6 | Whole exhaust | 1.8 | None | | 12 mo | 0/6 (0) | 0/6 (0) | 0/6 (0) | | | |
| | | NS, 13 | Whole exhaust | 1.8 | None | | 18 mo | 0/13 (0) | 1/13 (0) | 1/13 (0) | | | |
| Lewis et al. (1989) | Rat/F344 | M + F, 288[n] | Clean air | 0 | None | 7 h/day, | NA | No tumors | | 0/192 (0) | | | |
| | | | Whole exhaust | 2.0 | None | 5 days/week, 24 mo[o] | | | | 0/192 (0) | | | |
| | | | | | | | | Multiple adenomas | Multiple carcinomas | Adenomas/ carcinoma | Alveolar/ bronchiolar adenoma | Alveolar/ bronchiolar carcinoma | |
| Mauderly et al. (1996) | Mouse/ CD-1 | M + F | Clean air | 0 | None | 7 h/day, 5 | None | 1/157 (0.6) | 2/157 (1.3) | 1/157 (0.6) | 10/157 (6.4) | 7/157 (4.5) | |
| | | M + F | Whole exhaust | 0.35 | None | days/week, | | 2/171 (1.2) | 1/171 (0.6) | 1/171 (0.6) | 16/171 (9.4) | 5/171 (2.9) | |
| | | M + F | Whole exhaust | 3.5 | None | for up to 24 | | 0/155 (0) | 1/155 (0.6) | 0/155 (0) | 8/155 (5.2) | 6/155 (3.9) | |
| | | M + F | Whole exhaust | 7.0 | None | mo | | 0/186 (0) | 0/186 (0) | 0/186 (0) | 10/186 (5.4) | 4/186 (2.2) | |

[a]Table values indicate number with tumors/number examined (% animals with tumors).
[b]Number of animals examined for tumors.
[c]Significantly different from clean air controls.
[d]Diphenylnitrosamine; 6.25 mg/kg/week s.c. during first 25 weeks of exposure.
[e]Diphenylnitrasamine; 12.5 mg/kg/week s.c. during first 25 weeks of exposure.
[f]Splenic lymphomas also detected in controls (8.3%), filtered exhaust group (37.5%) and whole exhaust group (25%).
[g]5.3% incidence of large cell carcinomas.
[h]1 g/kg, i.p. 1/week for 3 weeks starting 1 mo into exposure.
[i]Includes adenomas, squamous cell carcinomas, adenocarcinomas, adenosquamous cell carcinoma, and mesotheliomas.
[j]4.5 mg/DEN/kg, s.c., 3 days prior to start of inhalation exposure.
[k]Single i.p. dose 1 mg/kg at start of exposure.
[l]Butylated hydroxytoluene 300 mg/kg, i.p. for week 1, 83 mg/kg for week 2, and 150 mg/kg for weeks 3 to 52.
[m]12 $mg/m^3$ from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents was 6 $mg/m^3$.
[n]120-121 males and 71-72 females examined histologically.
[o]Not all animals were exposed for full term, at least 10 males were killed at 3, 6, and 12 mo of exposure.
NS = Not specified.

NA = Not applicable.

respectively). Bronchoalveolar adenomas, adenocarcinomas, and squamous cysts (considered benign, except for two that were classified as squamous cell carcinomas because of the presence of less differentiated cells and invasion of blood and lymph vessels) were identified. Using the same statistical analysis of specific tumor types, adenocarcinoma plus squamous cell carcinoma and squamous cyst incidence was significantly greater in the high-exposure group, and the incidence of adenomas was significantly greater in the medium exposure group. A significant ($p$<0.001) exposure-response relationship was obtained for tumor incidence relative to exposure concentration and lung burden of DPM. These data are summarized in Table 7-1. A logistic regression model estimating tumor prevalence as a function of time, dose (lung burden of DPM), and sex indicated a sharp increase in tumor prevalence for the high dose level at about 800 days after the commencement of exposure. A less pronounced, but definite, increase in prevalence with time was predicted for the medium-dose level. Significant effects were not detected at the low concentration. DPM (mg per lung) of rats exposed to 0.35, 3.5, or 7.1 mg of DPM/$m^3$ for 24 mo were 0.6, 11.5, and 20.8, respectively, and affirmed the greater than predicted accumulation that was the result of decreased particle clearance following high-exposure conditions.

In summary, this study demonstrated the pulmonary carcinogenicity of high concentrations of whole, diluted diesel exhaust in rats following chronic inhalation exposure. In addition, increasing lung particle burden resulting from this high-level exposure and decreased clearance was demonstrated. A logistic regression model presented by Mauderly et al. (1987) indicated that both lung DPM burden and exposure concentration may be useful for expressing exposure-effect relationships.

A series of studies was conducted at the Fraunhofer Institute of Toxicology and Aerosol Research in which female Wistar rats were exposed for 19 h/day, 5 days/week to both filtered and unfiltered (total) diesel exhaust at an average particulate matter concentration of 4.24 mg/$m^3$. Animals were exposed for a maximum of 2.5 years. The exposure system as described by Heinrich et al. (1986b) used a 40 kw 1.6-L diesel engine operated continuously under the U.S. 72 FTP driving cycle. The engines used European Reference Fuel with a sulfur content of 0.36%. Filtered exhaust was obtained by passing engine exhaust through a Luwa FP-65 HT 610 particle filter heated to 80°C and a secondary series of filters (Luwa FP-85, Luwa NS-30, and Drager CH 63302) at room temperature. The filtered and unfiltered exhausts were each diluted 1:17 with filtered air and passed through respective 12 $m^3$ exposure chambers. Mass median aerodynamic diameter of DPM was 0.35 ± 0.10 µm (mean ± SD). The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A.

The effects of exposure to either filtered or unfiltered exhaust were described by Heinrich et al. (1986a) and Stöber (1986). Exposure to unfiltered exhaust resulted in 8 bronchoalveolar adenomas and 9 squamous cell tumors in 15 of 95 rats examined for a 15.8% tumor incidence.

Although statistical analysis was not provided, the increase appears to be highly significant. In addition to the bronchioalveolar adenomas and squamous cell tumors, there was a high incidence of bronchioalveolar hyperplasia (99%) and metaplasia of the bronchioalveolar epithelium (65%). No tumors were reported among female Wistar rats exposed to filtered exhaust (n = 92) or clean air (n = 96).

Mohr et al. (1986) provided a more detailed description of the lung lesions and tumors identified by Heinrich et al. (1986a, 1986b) and Stöber (1986). Substantial alveolar deposition of carbonaceous particles was noted for rats exposed to the unfiltered diesel exhaust. Squamous metaplasia was observed in 65.3% of the rats breathing unfiltered diesel exhaust but not in the control rats. Of nine squamous cell tumors, one was characterized as a Grade I carcinoma (borderline atypia, few to moderate mitoses, and slight evidence of stromal invasion), and the remaining eight were classified as benign keratinizing cystic tumors.

The effect of chronic (19 h/day, 5 days/week, 2 to 2.5 years) diesel exhaust exposure on the tumor-inducing effect of diphenylnitrosamine (DPN) was examined using female Wistar rats (Heinrich et al., 1986a; Stöber, 1986; Heinrich, 1989a). Groups of rats (45 to 48 per group) were exposed to clean air or whole diesel exhaust (particle concentration of 4.24 mg/m$^3$, as described previously) and administered by s.c. injection 250 or 500 mg DPN/kg/week during the first 25 weeks of exposure. The total DPN dose administered equaled 6.25 or 12.5 g/kg of body weight. The concentrations of B[a]P, benzo[e]pyrene (B[e]P), and chrysene in the diesel exhaust were 13, 21, and 76 ng/m$^3$, respectively.

The overall tumor rate in the lungs of DPN-treated rats was not affected by the exposure to either filtered or whole diesel engine exhaust. However, when only pulmonary squamous cell carcinomas were considered, the exposure to whole diesel exhaust significantly ($p \leq 0.05$) increased the tumor incidence (Table 7-1). Conversely, the high level of nasal tumors induced by DPN was significantly decreased in the rats exposed to the diesel engine emissions.

Heinrich et al. (1986b) and Mohr et al. (1986) compared the effects of exposure to particles having only a minimal carbon core but a much greater concentration of PAHs than DPM does. The desired exposure conditions were achieved by mixing coal oven flue gas with pyrolyzed pitch. The concentration of B[a]P and other PAHs per milligram of DPM was about three orders of magnitude greater than that of diesel exhaust. Female rats were exposed to the flue gas-pyrolyzed pitch for 16 h/day, 5 days/week at particle concentrations of 3 to 7 mg/m$^3$ for 22 mo, then held in clean air for up to an additional 12 mo. Among 116 animals exposed, 22 tumors were reported in 21 animals, for an incidence of 18.1%. One was a bronchioloalveolar adenoma, one was a bronchioloalveolar carcinoma, and 20 were squamous cell tumors. Among the latter, 16 were classified as benign keratinizing cystic tumors, and 4 were classified as

carcinomas. No tumors were reported in 115 controls. The tumor incidence in this study was comparable to that reported previously for the diesel exhaust-exposed animals.

The importance of DPM and of insoluble respirable particles in tumorigenic responses of rats was investigated and reported by Heinrich et al. (1995). In this chronic inhalation exposure study, female Wistar rats were exposed to whole diesel exhaust (0.8, 2.5, or 7.0 mg/m$^3$), 18 h/day, 5 days/week for up to 24 mo. Groups of rats were also exposed to ultrafine $TiO_2$ particles (10 mg/m$^3$) and carbon black particles (11.6 mg/m$^3$) using the same exposure regimen, except that after 4 mo the exposure concentrations of $TiO_2$ and carbon black were increased to obtain lung particle burdens similar to those observed in rats exposed to whole diesel exhaust. Controls were exposed to clean air. Following exposure to the test atmospheres, the rats were maintained in clean air atmospheres for an additional 6 mo.

In analyzing the studies of Heinrich et al. (1986a,b), Heinrich (1990), Mohr et al. (1986), and Stöber (1986), it must be noted that the incidence of lung tumors occurring following exposure to whole diesel exhaust, coal oven flue gas, or carbon black (15.8%, 18.1%, and 8% to 17%, respectively) was very similar. This occurred despite the fact that the PAH content of the PAH-enriched pyrolyzed pitch was more than three orders of magnitude greater than that of diesel exhaust; carbon black, on the other hand, had only traces of PAHs. Based on these findings, the organic fraction is not the sole cause of tumor induction by diesel exhaust. This issue is discussed further in Chapter 10.

In the Heinrich et al. (1995) report, the cumulative exposures for the rats in the various treatments groups were 61.7, 21.8, and 7.4 g/m$^3$ × h for the high, medium, and low whole-exhaust exposures and 102.2 and 88.1 g/m$^3$ × h for the carbon black and $TiO_2$ groups, respectively. For tumor incidence comparison (number of rats with tumors) among the diesel exhaust exposure groups, significant increases were observed in the high (22/100; $p<0.001$) and mid (11/200; $p<0.01$) exposure groups relative to clean air controls (Table 7-1). Only one tumor (1/217), an adenocarcinoma, was observed in clean air controls. Relative to clean air controls, significantly increased incidences were observed in the high exposure rats for benign squamous cell tumors (14/100; $p<0.001$), adenomas (4/100; $p<0.01$), and adenocarcinomas (5/100; $p<0.05$). Only the incidence of benign squamous cell tumors (7/200; $p<0.01$) was significantly increased in the mid-exposure group relative to the clean air controls. In comparing the number of rats with tumors (including benign squamous cell tumors), incidences in the $TiO_2$ exposure group (32/100) were similar to that of the high diesel exhaust exposure (22/100), but the incidence in the carbon black group (39/100) was significantly greater ($p<0.01$) than that of the high diesel exhaust. For the carbon black group, the incidences of adenomas (13/100) and adenocarcinomas (13/100) were significantly greater ($p<0.05$) than in the high diesel exhaust

group. The incidence of adenocarcinomas (13/100) was also significantly greater ($p<0.05$) in the $TiO_2$ group than in diesel exhaust-exposed rats.

Particle lung burden and alveolar clearance also were determined in the Heinrich et al. (1995) study. Even with adjustments to the exposure protocol to increase lung particle burden, at 12 mo of exposure and beyond the particle lung burden in high diesel exhaust group (63,878 µg/animal) was greater than that of the $TiO_2$ (43,854 µg/animal) and carbon black (39,287 µg/animal) groups. Relative to clean air controls, alveolar clearance was significantly compromised by exposure to mid and high diesel exhaust, $TiO_2$, and carbon black after 3 mo of exposure. Exposure to the high concentration of diesel exhaust resulted in a greater reduction of alveolar clearance than exposure to either $TiO_2$ or carbon black did. For the high diesel exhaust, $TiO_2$, and carbon black exposure groups, the 3-mo recovery time in clean air failed to reverse the compromised alveolar clearance.

A study conducted at the Inhalation Toxicology Research Institute also compared the lung tumor response of rats chronically exposed to diesel exhaust and carbon black (Nikula et al. 1995). In this study, male (114-115 per exposure group) and female (114-116 per exposure group) F344 rats were exposed to either carbon black or diesel exhaust for 16 h/day, 5 days/week to particle concentrations of 2.5 or 6.5 mg/m$^3$ for up to 24 mo. Controls (118 males, 114 females) were exposed to clean air. The progressive pulmonary accumulation of DPM tended to be more rapid than that for carbon black and accumulations of both tended to accelerate after 12 mo, a finding similar to that reported by Heinrich et al. (1994). At 23 mo, mean lung burdens of females exposed to low carbon black or high carbon black were 17.3 and 36.9 mg, respectively; and for males, the lung burdens were 24.7 and 40.1 mg, respectively. For low and high diesel exhaust exposure, the lung burdens were 36.7 and 80.7 mg, respectively, for females and 45.1 and 90.1 mg, respectively, for males. Both diesel exhaust and carbon black were pulmonary carcinogens under the exposure conditions of the study. The percentages of susceptible rats (males and females combined) with malignant neoplasms were 0.9 (control), 3.8 (low carbon black), 11.8 (high carbon black), 3.3 (low diesel exhaust), and 12.3 (high diesel exhaust). The percentages of rats (males and females combined) with malignant or benign neoplasms were 1.4 (control), 4.7 (low carbon black), 15.2 (high carbon black), 6.2 (low diesel exhaust), and 17.9 (high diesel exhaust). All primary neoplasms were associated with the parenchyma rather than the conducting airways of the lungs. The first lung neoplasm was observed at 15 mo. The specific tumor types and incidences are shown in Table 7-1. Analysis of the histopathologic data suggested a progressive process from alveolar epithelial hyperplasia to adenomas and adenocarcinomas. The neoplastic responses to carbon black and diesel exhaust were similar, indicating that the organic fraction adsorbed to the DPM was not contributing significantly to the carcinogenic response. Although these data provide indirect evidence that the DPM-associated

organic fraction of diesel exhaust did not play a significant role in the observed carcinogenic response, the data do not prove that the organic fraction has no role whatsoever. However, if DPM-associated organics are involved, the great difference in organic fraction content between carbon black and diesel exhaust (i.e., three orders of magnitude) suggests that its role was minor in the tumorigenic response of the rats in this study.

A long-term inhalation study (Ishinishi et al., 1988a; Takaki et al., 1989) examined the effects of emissions from light-duty (LD) and heavy-duty (HD) diesel engines on male and female Fischer 344/Jcl rats. The LD engines were 1.8-L, 4-cylinder, swirl-chamber-type power plants, and the HD engines were 11-L, 6-cylinder, direct-injection-type power plants. The engines were connected to eddy-current dynamometers and operated at 1,200 rpm (LD engines) and 1700 rpm (HD engines). Nippon Oil Co. JIS No. 1 or No. 2 diesel fuel was used. The 30-mo whole-body exposure protocol (16 h/day, 6 days/week) used DPM concentrations of 0, 0.5, 1, 1.8, or 3.7 $mg/m^3$ from HD engines and 0, 0.1, 0.4, 1.1, or 2.3 $mg/m^3$ from LD engines. The B[a]P concentrations were reported as 4.4 and 2.8 µg/g of particulate matter, and 1-nitropyrene concentrations were 57.1 and 15.3 µg/g of particulate matter for the LD and HD engines, respectively. An analysis of gas-phase components is presented in Appendix A. The animals inhaled the exhaust emissions from 1700 to 0900 h. Sixty-four male rats and 59 to 61 female rats from each exposure group were evaluated for carcinogenicity.

For the experiments using the LD series engines, the highest incidence of hyperplastic lesions plus tumors (72.6%) was seen in the highest exposure (2.3 $mg/m^3$) group. However, this high value was the result of the 70% incidence of hyperplastic lesions; the incidence of adenomas was only 0.8% and that of carcinomas 1.6%. Hyperplastic lesion incidence was considerably lower for the lower exposure groups (9.7%, 4.8%, 3.3%, and 3.3% for the 1.1, 0.4, and 0.1 $mg/m^3$ and control groups, respectively). The incidence of adenomas and carcinomas, combining males and females, was not significantly different among exposure groups (2.4%, 4.0%, 0.8%, 2.4%, and 3.3% for the 2.3, 1.1, 0.4, and 0.1 $mg/m^3$ groups and the controls, respectively).

For the experiments using the HD series engines, the total incidence of hyperplastic lesions, adenomas, and carcinomas was highest (26.6%) in the 3.7 $mg/m^3$ exposure group. The incidence of adenomas plus carcinomas for males and females combined equaled 6.5%, 3.3%, 0%, 0.8%, and 0.8% at 3.7, 1.8, 1, and 0.4 $mg/m^3$ and for controls, respectively. A statistically significant difference was reported between the 3.7 $mg/m^3$ and the control groups for the HD series engines. A progressive dose-response relationship was not demonstrated. Tumor incidence data for this experiment are presented in Table 7-1.

The Ishinishi et al. (1988a) study also included recovery tests in which rats exposed to whole diesel exhaust (DPM concentration of 0.1 or 1.1 $mg/m^3$ for the LD engine and 0.5 or

1.8 mg/m$^3$ for the HD engine) for 12 mo were examined for lung tumors following 6-, 12-, or 18-mo recovery periods in clean air. The incidences of neoplastic lesions were low, and pulmonary DPM burden was lower than for animals continuously exposed to whole diesel exhaust and not provided a recovery period. The only carcinoma observed was in a rat examined 12 mo following exposure to exhaust (1.8 mg/m$^3$) from the HD engine.

Iwai et al. (1986) also examined the long-term effects of diesel exhaust inhalation on female F344 rats. The exhaust was generated by a 2.4 L displacement truck engine. The exhaust was diluted 10:1 with clean air at 20°C to 25°C and 50% relative humidity. The engines were operated at 1,000 rpm with an 80% engine load. These operating conditions were found to produce exhaust with the highest particle concentration and lowest $NO_2$ and $SO_2$ content. For those chambers using filtered exhaust, proximally installed high-efficiency particulate air (HEPA) filters were used. Three groups of 24 rats each were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or filtered room air for 8 h/day, 7 days/week for 24 mo. Particle concentration was 4.9 mg/m$^3$ for unfiltered exhaust. Concentrations of gas-phase exhaust components were 30.9 ppm $NO_x$, 1.8 ppm $NO_2$, 13.1 ppm $SO_2$, and 7.0 ppm CO.

No lung tumors were found in the 2-year control (filtered room air) rats, although one adenoma was noted in a 30-mo control rat, providing a spontaneous tumor incidence of 4.5%. No lung tumors were observed in rats exposed to filtered diesel exhaust. Four of 14 rats exposed to unfiltered diesel exhaust for 2 years developed lung tumors, two of these were malignant. Five rats of this 2-year exposure group were subsequently placed in clean room air for 3 to 6 mo and four eventually (time not specified) exhibited lung tumors (three malignancies). Thus, the lung tumor incidence for total tumors was 42.1% (8/19) and 26.3% (5/19) for malignant tumors in rats exposed to whole diesel exhaust. The tumor types identified were adenoma (3/19), adenocarcinoma (1/19), adenosquamous carcinoma (2/19), squamous carcinoma (1/19), and large-cell carcinoma (1/19). The lung tumor incidence in rats exposed to whole diesel exhaust was significantly greater than that of controls ($p \leq 0.01$). Tumor data are summarized in Table 7-1. Malignant splenic lymphomas were detected in 37.5% of the rats in the filtered exhaust group and in 25.0% of the rats in the unfiltered exhaust group, these values were significantly ($p \leq 0.\ 05$) greater than the 8.2% incidence noted in the control rats. The study demonstrates production of lung cancer in rats following 2-year exposure to unfiltered diesel exhaust. In addition, splenic malignant lymphomas occurred during exposure to both filtered and unfiltered diesel exhaust. This is the only report to date of tumor induction at an extrarespiratory site by inhaled diesel exhaust in animals.

A chronic (up to 24 mo) inhalation exposure study by Takemoto et al. (1986) was conducted to determine the effects of diesel exhaust, di-isopropanol-nitrosamine (DIPN), and diesel exhaust following DIPN treatment of female F344/Jcl rats. One mo after initiation of

inhalation exposures, DIPN was administered i.p. at 1 mg/kg weekly for 3 weeks to clean air- and diesel-exposed groups of rats. Uninjected groups were also exposed to clean air and diesel exhaust. The treatment protocol consisted of exposure to diesel exhaust for 4 h/day, 4 days/week. The diesel exhaust was generated by a 269-cc engine operated at an idle state (1,600 rpm). Concentrations of the gas-phase components of the exhaust are presented in Appendix A. The particle concentration of the diesel exhaust in the exposure chamber was 2 to 4 mg/$m^3$. B[a]P and 1-nitropyrene concentrations were 0.85 and 93 µg/g of particles, respectively.

In the Takemoto et al. (1986) study, no lung tumors were reported in either uninjected controls or diesel-exposed animals. Among injected animals autopsied at 12 to 17 mo, 2 adenomas were reported in 8 rats exposed to clean air compared with 12 adenomas and 3 adenocarcinomas in 18 diesel-exposed rats. Among injected rats autopsied at 18 to 24 mo, 10 adenomas and 4 adenocarcinomas were seen in 21 animals exposed to clean air compared with 12 adenomas and 7 adenocarcinomas in 18 diesel-exposed rats. According to the authors, the incidence of malignant tumors was not significantly increased in either of the diesel exhaust-exposed groups when compared with the appropriate control group. Tumor incidence data for the various treatment protocols are presented in Table 7-1. It was also noted that the diesel engine employed in this study was originally used as an electrical generator and that its operating characteristics (not specified) were different from those for a diesel-powered automobile. However, the investigators deemed it suitable for assessing the of effects diesel emissions.

Brightwell et al. (1986, 1989) studied the effects of filtered and unfiltered diesel exhaust on male and female F344 rats. The diesel exhaust was generated by a 1.5-L Volkswagen engine that was computer-operated according to the U.S. 72 FTP driving cycle. The engine emissions were diluted by conditioned air delivered at 800 $m^3$/h to produce the high-exposure (6.6 mg/$m^3$) diesel exhaust atmosphere. Further dilutions of 1:3 and 1:9 produced the medium- (2.2 mg/$m^3$) and low- (0.7 mg/$m^3$) exposure atmospheres. Filtered diesel exhaust was generated by a similar engine. The CO and $NO_x$ concentrations (mean ± SD) were 32 ± 11 ppm and 8 ± 1 ppm for the unfiltered diesel exhaust (high-exposure concentration chamber) and 32 ± 11 and 8 ± 1 for the filtered diesel exhaust. The inhalation exposures were conducted overnight to provide five 16-h periods per week for 2 years; surviving animals were maintained for an additional 6 mo.

For males and females combined, a 9.7% (14/144) and 38.5% (55/143) incidence of primary lung tumors occurred in F344 rats following exposure to 2.2 and 6.6 mg of DPM/$m^3$, respectively (Table 7-1). The tumor incidence in the 0.7 mg/$m^3$ exposure group was 0.7% (1/144) and that of controls was 1.2% (3/260). Diesel exhaust-induced tumor incidence in rats was dose-related and higher in females than in males (Table 7-1). These data included animals sacrificed at the interim periods (6, 12, 18, and 24 mo); therefore, the tumor incidence does not accurately reflect the effects of long-term exposure to the diesel exhaust atmospheres. When

tumor incidence is expressed relative to the specific intervals, a lung tumor incidence of 96% (24/25), 76% (19/25) of which were malignant, was reported for female rats in the high dose group exposed for 24 mo and held in clean air for the remainder of their lives. For male rats in the same group, the tumor incidence equaled 44% (12/27), of which 37% (10/27) were malignant. It was also noted that many of the animals exhibiting tumors had more than one tumor, often representing multiple histological types. The types of tumors identified in the rats exposed to diesel exhaust included adenomas, squamous cell carcinomas, adenocarcinomas, mixed adenoma/adenocarcinomas, and mesotheliomas. Similar to other studies, the tumor incidence in rats occurred during exposure to whole exhaust rather than filtered exhaust. It must be noted, however, that the exposure during darkness (when increased activity would result in greater respiratory exchange and greater inhaled dose) could account, in part, for the high response reported for the rats.

Karagianes et al. (1981) exposed male Wistar rats (40 per group) to diesel engine exhaust diluted to a DPM concentration of 8.3 (± 2.0) mg/m$^3$, room air, diesel engine exhaust (8.3 mg/m$^3$) plus low-concentration coal dust (5.8 mg/m$^3$), low-concentration coal dust only (6.6 mg/m$^3$), or high-concentration coal dust (14.9 mg/m$^3$) 6 h/day, 5 days/week for up to 20 mo. The exhaust-generating system and exposure atmosphere characteristics are presented in Appendix A. The type of engine used (3-cylinder, 43 bhp diesel) is normally used in mining situations and was connected to an electric generator and operated at varying loads and speeds to simulate operating conditions in an occupational situation. To control the CO concentration at 50 ppm, the exhaust was diluted 35:1 with clean air.

One bronchiolar adenoma was detected in the group exposed to diesel exhaust alone and one in the rats receiving combined exposures. No lung tumors were reported in controls or following exposure to either high or low concentrations of coal dust. The equivocal response may have been caused by the relatively short exposure durations (20 mo). In the Mauderly et al. (1987) study, by comparison, most of the tumors were detected in rats exposed for more than 24 mo.

Lewis et al. (1989) also examined the effects of inhalation exposure of diesel exhaust and/or coal dust on tumorigenesis on F344 rats. Groups of 216 male and 72 female rats were exposed to clean air, whole diesel exhaust (2 mg soot/m$^3$), coal dust (2 mg/m$^3$ respirable concentration; 5 to 6 mg/m$^3$ total concentration), or diesel exhaust plus coal dust (1 mg/m$^3$ of each respirable concentration; 3.2 mg/m$^3$ total concentration) for 7 h/day, 5 days/week for up to 24 mo. Groups of 10 or more males were sacrificed at intermediate intervals (3, 6, and 12 mo). The diesel exhaust was produced by a 7.0-L, 4-cycle, water-cooled Caterpillar Model 3304 engine using No. 2 diesel fuel (<0.5% sulfur by mass). The exhaust was passed through a Wagner water

scrubber, which lowered the exhaust temperature and quenched engine backfire. An analysis of the exposure atmospheres is presented in Appendix A.

Histological examination was performed on 120 to 121 male and 71 to 72 female rats terminated after 24 mo of exposure. The exhaust exposure did not significantly affect the tumor incidence beyond what would be expected for aging F344 rats. There was no postexposure period, which may explain, in part, the lack of significant tumor induction. The particulate matter concentration was also less than the effective dose in several other studies.

General Motors Research Laboratories sponsored chronic inhalation studies using male Fischer 344 rats exposed to DPM concentrations of 0.25, 0.75, or 1.5 mg/m$^3$ (Kaplan et al., 1983; White et al., 1983). The exposure protocol for this study conducted at the Southwest Research Institute was 20 h/day, 7 days/week for 9 to 15 mo. Some animals were sacrificed following completion of exposure, while others were returned to clean air atmospheres for an additional 8 mo. Control animals received clean air. Exhaust was generated by 5.7-L Oldsmobile engines (four different engines used throughout the experiment) operated at a steady speed and load simulating a 40-mph driving speed of a full-size passenger car. Details of the exhaust-generating system and exposure atmosphere are presented in Appendix A.

Five instances of bronchoalveolar carcinoma were observed in 90 rats exposed to diesel exhaust for 15 mo and held an additional 8 mo in clean air. These included one tumor in the 0.25 mg/m$^3$ group, three in the 0.75 mg/m$^3$ group, and one in the 1.5 mg/m$^3$ group. Rats kept in clean air chambers for 23 mo did not exhibit any carcinomas. No tumors were observed in any of the 180 rats exposed to diesel exhaust for 9 or 15 mo without a recovery period or in the respective controls for these groups. Although the increases in tumor incidences in the groups exposed for 15 mo and held an additional 8 mo in clean air were not statistically significant, they suggest an effect because the background incidence for this specific lesion in this strain of rat is low.

### 7.2.2. Mouse Studies

Heinrich et al. (1986a) and Stöber (1986), as part of a larger study, also evaluated the effects of diesel exhaust in mice. Details of the exposure conditions reported by Heinrich et al. (1986b) are given in Appendix A. Following lifetime (19 h/day, 5 days/week, for a maximum of 120 weeks) exposure to filtered (n = 93) and unfiltered (n = 76) diesel exhaust (4.2 mg/m$^3$), female NMRI mice exhibited a total lung tumor incidence of adenomas and adenocarcinomas combined of 31% (filtered) and 32% (unfiltered), respectively. Tumor incidences reported for control mice (n = 84) equaled 11% for adenomas and adenocarcinomas combined. The effects are more dramatic when the incidences of only malignant tumors (adenocarcinomas) are considered, 2.4% for controls, 19% for filtered exhaust, and 17% for unfiltered exhaust. This is

the only reported study in which filtered exhaust resulted in a definitive tumorigenic response in the lungs of mice. These data are summarized in Table 7-1.

As part of the same study, groups of 64 female NMRI mice of 8 to 10 weeks of age were dosed weekly with either 50 or 100 µg B[a]P intratracheally for 20 or 10 weeks, respectively, for a total dose of 1 mg. Another group received 50 µg dibenz[*a,h*]anthracene (DBA) intratracheally for 10 weeks. Additional groups of 96 newborn mice received one s.c. injection of 5 or 10 µg of DBA between 24 and 48 h after birth. The animals were concomitantly exposed to either diesel exhaust or clean air. The mice receiving intratracheal instillations were observed throughout their lifespan but the newborn mice were sacrificed after 6 mo. Although the chemical treatments resulted in large increases in lung tumor incidence, exposure to diesel exhaust did not enhance this effect and in some cases even resulted in inhibition. For example, lung tumor rates in clean air mice treated with 20 instillations of B[a]P equaled 71% compared with 41% for mice similarly instilled but exposed to diesel exhaust. The decrease resulted from a smaller number of adenocarcinomas, whereas the adenoma incidence remained unchanged. The high dose of DBA injected into newborn mice also resulted in a greater tumor incidence in mice exposed to clean air (81%) than in the diesel exposed group (63%). Effects of the other treatments were apparently not inhibited by diesel exhaust exposure, although complete incidence data were not reported. The authors did not speculate on the reasons for this unexpected effect.

In addition to the studies using rats, investigators at the Fraunhofer Institute also examined the effects in mice following long-term exposure to whole diesel exhaust, $TiO_2$, and carbon black particles (Heinrich et al., 1995). In these studies, NMRI mice were exposed (18 h/day, 5 days/week) to whole diesel exhaust (4.5 mg/m$^3$ for 13.5 mo), $TiO_2$ (7.0 mg/m$^3$ for 4 mo followed by 15.0 mg/m$^3$ for 4 mo and 10 mg/m$^3$ for 5.5 mo), or carbon black (7.0 mg/m$^3$ for 4 mo followed by 12 mg/m$^3$ for 9.5 mo). Following the 13.5-mo exposures, animals were kept in clean air for a total experiment time of 23 mo. Controls were exposed to clean air. The lung burdens of the NMRI mice after 12 mo of exposure were 7.0, 7.4, and 5.2 mg/lung for DPM, carbon black, and $TiO_2$, respectively. Adenomas and adenocarcinomas were the only tumor types observed in the mice. Percentages for adenomas/adenocarcinomas were 21.8%/15.4% for whole diesel exhaust, 11.3%/10% for carbon black, 11.3%/2.5% for $TiO_2$, and 25%/15.4% for clean air controls. The lung tumor rates for adenomas/adenocarcinomas were 32.1% (diesel exhaust), 20% (carbon black), 13.8% ($TiO_2$), and 30% (clean air controls).

A comparison between particle-free exhaust and whole exhaust was also conducted using NMRI and C57BL mice (Heinrich et al., 1995). In the experiments with NMRI mice, mice were exposed to whole diesel exhaust (4.5 mg/m$^3$) and another group was exposed to particle-free diesel exhaust for 23 mo. The lung tumor rates for the groups of mice were 23% (whole exhaust), 46.7% (particle-free exhaust), and 30% (clean air controls). The difference between the

controls and those exposed to the particle-free exhaust was not significant ($p$=0.053). The percentages of adenomas/adenocarcinomas were 18.3%/5% (whole exhaust), 31.7%/15% (particle-free exhaust), and 25%/8.8% (clean air controls). In the experiments using C57BL mice, the exposure groups were similar except that the exposure duration was 24 mo. The tumor rates (including an additional 6-mo exposure in clean air) were not significantly different among the whole exhaust (8.5%), particle-free exhaust (3.5%), and clean air controls (5.1%).

The lack of a carcinogenic response in mice was reported by Mauderly et al. (1996). In this study, groups of 540 to 600 CD-1 male and female mice were exposed to whole diesel exhaust (7.0, 3.5, or 0.35 mg DPM/$m^3$) for 7 h/day, 5 days/week for up to 24 mo. Controls were exposed to filtered air. DPM accumulation in the lungs of exposed mice was assessed at 6, 12, and 18 mo of exposure and was shown to be progressive; DPM burdens were 0.2 ± 0.02, 3.7 ± 0.16, and 5.6 ± 0.39 mg for the low-, medium-, and high-exposure groups, respectively. The lung burdens in both the medium- and high-exposure groups exceeded that predicted by exposure concentration ratio to the low-exposure group. Contrary to what was observed in rats (Heinrich et al., 1986a; Stöber, 1986; Nikula et al., 1995; Mauderly et al., 1987), an exposure-related increase in primary lung neoplasms was not observed in the CD-1 mice, supporting the contention of a species difference in the pulmonary carcinogenic response to poorly soluble particles. The percentage incidence of mice (males and females combined) with one or more malignant or benign neoplasms was 13.4, 14.6, 9.7, and 7.5 for controls and low-, medium-, and high-exposure groups, respectively.

Takemoto et al. (1986) reported the effects of inhaled diesel exhaust (2 to 4 mg/$m^3$, 4 h/day, 4 days/week, for up to 28 mo) in ICR and C57BL mice exposed from birth. Details of the exposure conditions are presented in Appendix A. Among male and female ICR mice autopsied at 13 to 18 mo, 4 adenomas and 1 adenocarcinoma were detected in 34 diesel exhaust-exposed mice compared with 3 adenomas among 38 controls. Among animals autopsied at 19 to 28 mo, 6 adenomas and 3 adenocarcinomas were seen in 22 exposed animals compared with 3 adenomas and one adenocarcinoma in 22 controls. Among combined male and female C57BL mice autopsied at 13 to 18 mo, 4 adenomas and 2 adenocarcinomas were detected in 79 animals autopsied compared with none in 19 unexposed animals. Among males and females autopsied at 19 to 28 mo, 8 adenomas and 3 adenocarcinomas were detected in 71 exposed animals compared with 1 adenoma among 32 controls. No significant increases in either adenoma or adenocarcinoma incidences were reported for either strain of exposed mice. Although not tested by the authors, the combined incidence of adenomas and adenocarcinomas (11/71) in male and female C57BL mice exposed to diesel exhaust for 19 to 28 mo versus that found in controls (1/32), however, appears to be a significant increase. Although the results are not definitive, there

is the strong suggestion of an effect, especially since the C57BL strain has a low background lung tumor incidence. See Table 7-1 for details of tumor incidence.

Pepelko and Peirano (1983) summarized a series of studies on the health effects of diesel emissions in mice. Exhaust was provided by two Nissan CN 6-33, 6-cylinder, 3.24-L diesel engines coupled to a Chrysler A-272 automatic transmission and Eaton model 758-DG dynamometer. Details of the exhaust-generating system and the exposure atmosphere are presented in Appendix A. Sixty-day pilot studies were conducted at a 1:14 dilution, providing DPM concentrations of 6 mg/$m^{3.}$ The engines were operated using the Modified California Cycle. These 20-h/day, 7-days/week pilot studies using rats, cats, guinea pigs, and mice produced decreases in weight gain and food consumption. Therefore, at the beginning of the long-term studies, exposure time was reduced to 8 h/day, 7 days/week at an exhaust DPM concentration of 6 mg/$m^3$. During the final 12 mo of exposure, however, the DPM concentration was increased to 12 mg/$m^3$. For the chronic studies, the engines were operated using the Federal Short Cycle.

Pepelko and Peirano (1983) described a two-generation study using Sencar mice exposed to diesel exhaust alone or treated with either tumor initiators or promoters. Male and female parent generation mice were exposed to diesel exhaust at a DPM concentration of 6 mg/$m^3$ prior to (from weaning to sexual maturity) and throughout mating. The dams continued exposure through gestation, birth, and weaning. Groups of offspring (130 males and 130 females) received i.p. injections of either butylated hydroxytoluene (BHT) (300 mg/kg for week 1, 83 mg/kg for week 2, and 150 mg/kg from week 3 to 1 year), a single i.p. injection of 1 mg urethan at 6 weeks of age, or no injections. The exhaust exposure was increased to a DPM concentration of 12 mg/$m^3$ when the offspring were 12 weeks of age and was maintained until termination of the experiment when the mice were 15 mo old.

The incidence of pulmonary adenomas (16.3%) was significantly increased in the non-injected female mice exposed to diesel exhaust compared with 6.3% in clean air controls. The incidence in males and females combined was 10.2% in 205 animals examined compared with 5.1% in 205 clean air controls. This difference was also significant. The incidence of carcinomas was not affected by exhaust exposure in either sex. Exhaust exposure reduced the adenoma incidence in female mice receiving BHT (3.9% vs. 16.7%). The response to BHT in males or urethan in both sexes was unaffected by diesel exposure. These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The limited response may well have been influenced by the relatively early sacrifice times of the mice. On the other hand, an increase in the sensitivity of the study, allowing detection of tumors at 15 mo, may have been the result of exposure from conception. It is interesting to note that in this study diesel exposure appeared to inhibit effects of tumor promotion, whereas Stöber (1986) reported diesel exposure inhibition of complete carcinogens. These data are summarized in Table 7-1.

A series of inhalation studies using strain A mice was conducted by Orthoefer et al. (1981). In assays with the strain A, mice are usually given a series of intraperitoneal injections with the test agent; they are then sacrificed at about 9 mo of age and examined for lung tumors. In the present series, inhalation exposure was substituted. In the current series, groups of 25 male Strong A mice were exposed to irradiated (to simulate chemical reactions induced by sunlight) or nonirradiated diesel exhaust (6 mg/m$^3$) for 20 h/day, 7 days/week for 7 weeks. Additional groups of 40 Jackson A (20 of each sex) were exposed similarly to either clean air or diesel exhaust, then held in clean air until sacrificed at 9 mo of age. No tumorigenic effects were detected at 9 mo of age. Further studies were conducted in which male A/Strong mice were exposed 8 h/day, 7 days/week until sacrifice (approximately 300 at 9 mo of age and approximately 100 at 12 mo of age). With the exception of those treated with urethan, the number of tumors per mouse did not exceed historical control levels in any of the studies. Exposure to diesel exhaust, however, significantly inhibited the tumorigenic effects of the 5-mg urethan treatment. Results are listed in Table 7-1.

Kaplan et al. (1982) also reported the effects of diesel exposure in strain A mice. Groups of male strain A/J mice were exposed for 20 h/day, 7 days/week for 90 days and held until 9 mo of age. Experimental conditions are described in Appendix A. Briefly, the animals were exposed to diesel exhaust at DPM concentrations of 0, 0.25, 0.75, or 1.5 mg/m$^3$. Controls were exposed to clean air. Among 458 controls and 485 exposed animals, tumors were detected in 31.4% of those breathing clean air versus 34.2% of those exposed to diesel exhaust. The mean number of tumors per mouse also failed to show significant differences.

In a follow-up study, strain A mice were exposed to diesel exhaust for 8 mo (Kaplan et al., 1983; White et al., 1983). After exposure to the highest exhaust concentration (1.5 mg/m$^3$), the percentage of mice with pulmonary adenomas and the mean number of tumors per mouse were significantly less ($p$<0.05) than those for controls (25.0% vs. 33.5% and 0.30 ± 0.02 [S.E.] vs. 0.42 ± 0.03 [S.E.]) (Table 7-1).

### 7.2.3. Hamster Studies

Heinrich et al. (1982) examined the effects of diesel exhaust exposure on the tumor frequency in female Syrian golden hamsters pretreated with the tumor initiators dibenzo[a,h]anthracene (DBA) or diethylnitrosamine (DEN). At the time of this work, it was presumed that traditional inhalation exposure experiments would not result in definitive tumor formation; thus a tumor initiation animal model was used. Groups of 48 to 72 animals were exposed to clean air, whole diesel exhaust at a mean DPM concentration of 3.9 mg/m$^3$, or filtered diesel exhaust with either no further treatment or administered DBA (itr. instillations of 0.1 mg/week for 20 weeks), DEN (1.5 or 4.5 mg/kg s.c.), or pyrene (itr. instillations of 0.1 mg/week

for 20 weeks), the last serving as a noncarcinogenic PAH control. Inhalation exposures were conducted 7 to 8 h/day, 5 days/week for 2 years. The exhaust was produced by a 2.4-L Daimler-Benz engine operated at 2,400 rpm.

Only two hamsters exhibited lung tumors, both having died during the exposure period. One tumor occurred in a hamster receiving DBA and exposed to filtered diesel exhaust for 75 weeks; the other tumor occurred in a hamster receiving DEN and exposed to whole diesel exhaust for 67 weeks. Compared with corresponding treatment groups, there was a higher incidence of adenomatous proliferative changes in the lungs of hamsters exposed to whole diesel exhaust. Hamsters exposed to filtered diesel exhaust also showed a greater incidence of adenomatous proliferative changes than those of the respective clean air exposure groups did. The incidence of proliferative changes in the lungs of hamsters receiving DEN or DBA was greater than for those groups not treated with the initiators. Although not definitive, this study provided information suggesting the possible involvement of whole diesel exhaust and filtered diesel exhaust in producing histologic alterations in the lungs of hamsters, though no increases in tumors were observed.

In a more recent study, Syrian hamsters were exposed 19 h/day, 5 days/week for a lifetime to diesel exhaust diluted to a DPM concentration of 4.24 mg/m$^3$ (Heinrich et al., 1986a; Stöber, 1986). Details of the exposure conditions are reported in Appendix A. Ninety-six animals per group were exposed to clean air, whole exhaust, or filtered exhaust. Additional groups were treated with DEN (4.5 mg/kg, s.c.) or B[a]P (20 doses of 0.25 mg itr. instillation) and exposed to the three experimental atmospheres. No lung tumors were seen in the uninjected clean air group or in either diesel exhaust-exposed group. Initial treatment with DEN or B[a]P resulted in lung tumor incidences of only 10% and 2%, respectively, which were not significantly changed by exposure to diesel exhaust.

Heinrich et al. (1989b) reported results of experiments assessing the effects of DEN and diesel exhaust exposure in combination. Hamsters were exposed to exhaust from a Daimler-Benz 2.4-L engine operated at a constant load of about 15 kW and at a uniform speed of 2,000 rpm. The exhaust was diluted to an exhaust-clean air ratio of about 1:13, resulting in a mean particle concentration of 3.75 mg/m$^3$. The animals were exposed 19 h/day, 5 days/week beginning at noon each day, under a 12-h light cycle starting at 7 a.m. DEN (3 or 6 mg/kg) was given as a single s.c. injection 2 weeks from the start of exposure to groups of 40 male and 40 female Syrian golden hamsters exposed to whole diesel exhaust, filtered diesel exhaust, or clean air. Groups were also exposed to the exhaust without DEN or to only clean air. Exposures were conducted in chambers maintained at 22°C to 24°C and 40% to 60% relative humidity for up to 18 mo. Surviving hamsters were maintained in clean air for up to an additional 6 mo. The concentrations of B[a]P and B[e]P in the whole exhaust atmospheres were 37.5 and 61.9 ng/m$^3$, respectively.

No lung tumors were detected in any of the treatment groups. A nasal carcinoma was detected in a female hamster treated with DEN (6 mg/kg) and exposed to filtered exhaust. A tracheal carcinoma was detected in a male hamster exposed to whole diesel exhaust and receiving DEN (3 mg/kg), and a laryngeal carcinoma was observed in a male hamster receiving DEN (6 mg/kg) and exposed to whole diesel exhaust. Exposure of male hamsters to whole or filtered diesel exhaust alone did not result in a significant increase in the tumors relative to clean air controls. Male hamsters receiving 6 mg DEN/kg plus whole diesel exhaust exposure and dying before or after the 50% survival date, however, did show an increase in tumor rate compared with DEN-treated animals exposed to clean air. Using life-table analysis, a significant ($p<0.05$) exposure-related increase in tumor rate was noted for this group (40.0% vs. 7.0% for filtered exhaust + DEN and 7.0% for clean air + DEN). No upper respiratory tract tumors were detected in clean air controls or filtered-exhaust-exposed groups that did not receive the DEN treatment.

In summary, diesel exhaust alone did not induce an increase in lung tumors in hamsters of either sex. Diesel exhaust significantly enhanced the tumorigenic effects of DEN in males injected with 6 mg DEN/kg but not in females or in either sex given the 3 mg/kg dose. The co-carcinogenic effects of diesel with DEN therefore appear to be equivocal. It should be noted that the tumors occurred in the upper airways as opposed to the alveolar region in rats.

Brightwell et al. (1986, 1989) studied the effects of filtered or unfiltered diesel exhaust on male and female Syrian golden hamsters. Groups of 52 males and 52 females received no injections or s.c. injections of 4.5 mg DEN/kg 3 days before the start of exposure. The animals were 6 to 8 weeks old at the start of exposure to diesel exhaust at DPM concentrations of 0.7, 2.2, or 6.6 mg/m$^3$. They were exposed 16 h/day, 5 days/week for a total of 2 years and then sacrificed. Exposure conditions are described in Appendix A. Although the DEN-pretreated hamsters exhibited an increase in tracheal papillomas in all treatment groups when compared with non-DEN pretreated hamsters, there was no statistically significant (*t* test) relationship between tumor incidence and exhaust exposure. As noted in IARC (1989), however, the reporting of tumor incidence and survival was incomplete.

### 7.2.4. Monkey Studies

Fifteen male cynomolgus monkeys were exposed to diesel exhaust (2 mg/m$^3$) for 7 h/day, 5 days/week for 24 mo (Lewis et al., 1989). The same numbers of animals were also exposed to coal dust (2 mg/m$^3$ respirable concentration; 5 to 6 mg/m$^3$ total concentration), diesel exhaust plus coal dust (1 mg/m$^3$ respirable concentration for each component; 3.2 mg/m$^3$ total concentration), or filtered air. Details of exposure conditions were listed previously in the description of the Lewis et al. (1986) study with rats (Appendix A).

None of the monkeys exposed to diesel exhaust exhibited a significantly increased incidence of preneoplastic or neoplastic lesions. It should be noted, however, that the 24-mo timeframe employed in this study may not have allowed the manifestation of tumors in primates, because this duration is only a small fraction of the monkeys' expected lifespan. In fact, there have been no near-lifetime exposure studies in nonrodent species.

## 7.3. LUNG IMPLANTATION OR INTRATRACHEAL INSTILLATION STUDIES

### 7.3.1. Rat Studies

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that the PAHs in diesel exhaust that consist of four or more rings have a carcinogenic potential. Condensate was obtained from the whole exhaust of a 3.0 L passenger-car diesel engine connected to a dynamometer operated under simulated city traffic driving conditions. This condensate was separated by liquid-liquid distribution into hydrophilic and hydrophobic fractions representing 25% and 75% of the total condensate, respectively. The hydrophilic, hydrophobic, or reconstituted hydrophobic fractions were surgically implanted into the lungs of the rats. Untreated controls, vehicle (beeswax/trioctanoin) controls, and positive (B[a]P) controls were also included in the protocol (Table 7-2). Fraction Ilb (made up of PAHs

**Table 7-2. Tumor incidence and survival time of rats treated with fractions from diesel exhaust condensate (35 rats/group)**

| Material portion by weight (%) | Dose (mg) | Median survival time in weeks (range) | Number of carcinomas[a] | Number of adenomas[b] | Carcinoma incidence (%) |
|---|---|---|---|---|---|
| Hydrophilic fraction (I) (25) | 6.70 | 97 (24-139) | 0 | 1 | 0 |
| Hydrophobic fraction (II) (75) | 20.00 | 99 (50-139) | 5 | 0 | 14.2 |
| Nonaromatics + | | | | | |
| PAC[c] 2 + 3 rings (IIa) (72) | 19.22 | 103 (25-140) | 0 | 1 | 0 |
| PAH[d] 4 to 7 rings (IIb) (0.8) | 0.21 | 102 (50-140) | 6 | 0 | 17.1 |
| Polar PAC (IIc) (1.1) | 0.29 | 97 (44-138) | 0 | 0 | 0 |
| Nitro-PAH (IId) (0.7) | 0.19 | 106 (32-135) | 1 | 0 | 2.8 |
| Reconstituted hydrophobics (Ia, b, c, d) (74.5) | 19.91 | 93 (46-136) | 7 | 1 | 20.0 |
| Control, unrelated | | 110 (23-138) | 0 | 0 | 0 |
| Control (beeswax/trioctanoin) | | 103 (51-136) | 0 | 1 | 0 |
| Benzo[*a*]pyrene | 0.3 | 69 (41-135) | 27 | 0 | 77.1 |
| | 0.1 | 98 (22-134) | 11 | 0 | 31.4 |
| | 0.03 | 97 (32-135) | 3 | 0 | 8.6 |

[a]Squamous cell carcinoma.
[b]Bronchiolar/alveolar adenoma.
[c]PAC = polycyclic aromatic compounds.
[d] PAH = polycyclic aromatic hydrocarbons.

Source: Adapted from Grimmer et al. (1987).

with four to seven rings), which accounted for only 0.8% of the total weight of DPM condensate, produced the highest incidence of lung carcinomas following implantation into the rat lungs. A carcinoma incidence of 17.1% was observed following implantation of 0.21 mg IIb/rat, whereas the nitro-PAH fraction (IId) at 0.18 mg/rat accounted for only a 2.8% carcinoma incidence. Hydrophilic fractions of the DPM extracts, vehicle (beeswax/trioctanoin) controls, and untreated controls failed to exhibit carcinoma formation. Administration of all hydrophobic fractions (IIa-d) produced a carcinoma incidence (20%) similar to the summed incidence of fraction IIb (17.1%) and IId (2.8%). The B[a]P positive controls (0.03, 0.1, 0.3 mg/rat) yielded a carcinoma incidence of 8.6%, 31.4%, and 77.1%, respectively. The study showed that the tumorigenic agents were primarily four- to seven-ring PAHs and, to a lesser extent, nitroaromatics. However, these studies demonstrated that simultaneous administration of various PAH compounds resulted in a varying of the tumorigenic effect, thereby implying that the tumorigenic potency of PAH mixtures may not depend on any one individual PAH. This study did not provide any information regarding the bioavailability of the particle-associated PAHs that might be responsible for carcinogenicity.

Kawabata et al. (1986) compared the effects of activated carbon and diesel exhaust on lung tumor formation. One group of 59 F344 rats was intratracheally instilled with DPM 1 mg/week for 10 weeks). A second group of 31 rats was instilled with the same dosing regime of activated carbon. Twenty-seven rats received only the solvent (buffered saline with 0.05% Tween 80), and 53 rats were uninjected. Rats dying after 18 mo were autopsied. All animals surviving 30 mo or more postinstillation were sacrificed and evaluated for histopathology. Among 42 animals exposed to DPM surviving 18 mo or more, tumors were reported in 31, including 20 malignancies. In the subgroup surviving for 30 mo, tumors were detected in 19 of 20 animals, including 10 malignancies. Among the rats exposed to activated carbon, the incidence of lung tumors equaled 11 of 23 autopsied, with 7 cases of malignancy. Data for those dying between 18 and 30 mo and those sacrificed at 30 mo were not reported separately. Statistical analysis indicated that activated carbon induced a significant increase in lung tumor incidence compared with no tumors in 50 uninjected controls and 1 tumor in 23 solvent-injected controls. The tumor incidence increase was significant in the DPM-instilled group and was significantly greater than the increase in the carbon-instilled group. This study provides evidence for the carcinogenicity of DPM. It also shows, as Heinrich et al. (1995) and Nikula et al. (1995) did, that particles lacking organic constituents also can induce tumors.

Dasenbrock et al. (1996) conducted a study to determine the relative importance of the organic constituents of diesel particles and particle surface area in the induction of lung cancer in rats. Fifty-two female Wistar rats were intratracheally instilled with 16-17 doses of diesel particles (DPM), extracted DPM, printex carbon black (PR), lampblack (LB), benzo[*a*]pyrene

(B*a*P), DPM + B*a*P, or PR + B*a*P. The animals were held for a lifetime or sacrificed when moribund. The lungs were necropsied and examined for tumors. Diesel particles were collected from a Volkswagen 1.6 L engine operating on a US FTP-72 driving cycle. The mass median aerodynamic diameter (MMAD) of the diesel particles was 0.25 µm and the specific surface area was 12 $m^2$/gm. Following extraction with toluene MMAD increased to 138 $m^2$/gm. The MMAD for extracted PR was equal to 14 nm, while the surface area equaled 271 $m^2$/gm. The MMAD for extracted lampblack was equal to 95 nm, with a surface area equal to 20 $m^2$/gm. The B*a*P content of the treated particles was 11.3 mg per gm diesel particles and 29.5 mg B*a*P per gm PR. Significant increases in lung tumors were detected in rats instilled with 15 mg unextracted DPM and 30 mg extracted DPM, but not 15 mg extracted DPM. Printex CB was more potent than lamplack CB for induction of lung tumors, while B*a*P was effective only at high doses. Total dose and tumor responses are shown in Table 7-3.

A number of conclusions can be drawn from these results. First of all, particles devoid of organics are capable of inducing lung tumor formation, as indicated by positive results in the groups treated with high dose extracted diesel particles and printex. Nevertheless, extraction of organics from diesel particles results in a decrease in potency, indicating that the organic fraction does play a role in cancer induction. A relationship between cancer potency and particle surface area was also suggested by the finding that printex with a large specific surface area was more potent than either extracted DPM or lampblack, which have smaller specific areas. Finally, while very large doses of B*a*P are very effective in the induction of lung tumors, smaller doses adsorbed to particles surfaces had little detectable effect, suggesting that other organic components of DE may be of greater importance in the induction of lung tumors.

### 7.3.2. Syrian Hamster Studies

Kunitake et al. (1986) and Ishinishi et al. (1988b) conducted a study in which total doses of 1.5, 7.5, or 15 mg of a dichloromethane extract of DPM were instilled intratracheally over 15 weeks into male Syrian hamsters that were then held for their lifetimes. The tumor incidences of 2.3% (1/44), 0% (0/56), and 1.7% (1/59) for the high-, medium-, and low-dose groups, respectively, did not differ significantly from the 1.7% (1/56) reported for controls. Addition of 7.5 mg of B[a]P to a DPM extract dose of 1.5 mg resulted in a total tumor incidence of 91.2% and malignant tumor incidence of 88%. B[a]P (7.5 mg over 15 weeks) alone produced a tumor incidence rate of 88.2% (85% of these being malignant), which was not significantly different from the DPM extract + B[a]P group. Intratracheal administration of 0.03 µg B[a]P, the equivalent content in 15 mg of DPM extract, failed to cause a significant increase in tumors in

**Table 7-3. Tumor incidences in rats following intratracheal instillation of diesel exhaust particles (DPM), extracted DPM, carbon black (CB), benzo[*a*]pyrene (B*a*P), or particles plus B*a*P**

| Experimental group | Number of animals | Total, dose | Animals with tumors (percent) | Statistical significance[a] |
|---|---|---|---|---|
| Control | 47 | 4.5 mL | 0 (0) | - |
| DPM (original) | 48 | 15 mg | 8 (17) | < 0.01 |
| DPM (extracted) | 48 | 30 mg | 10 (21) | < 0.001 |
| DPM (extracted) | 48 | 15 mg | 2 (4) | NS |
| CB (printex) | 48 | 15 mg | 10 (21) | < 0.001 |
| CB (lampblack) | 48 | 14 mg | 4 (8) | NS |
| B*a*P | 47 | 30 mg | 43 (90) | < 0.001 |
| B*a*P | 48 | 15 mg | 12 (25) | < 0.001 |
| DEP + B*a*P | 48 | 15 mg 170 µg B*a*P | 4 (8) | NS |
| CB (printex) + B*a*P | 48 | 15 mg 443 µg B*a*P | 13 (27) | < 0.001 |

[a]Fischer's exact test.

rats. This study demonstrated a lack of detectable interaction between DPM extract and B[a]P, the failure of DPM extract to induce carcinogenesis, and the propensity for respiratory tract carcinogenesis following intratracheal instillation of high doses of B[a]P. For studies using the DPM extract, some concern must be registered regarding the known differences in chemical composition between DPM extract and DPM. As with all intratracheal instillation protocols, DPM extract lacks the complement of volatile chemicals found in whole diesel exhaust.

The effects on hamsters of intratracheally instilled DPM suspension, DPM with $Fe_2O_3$, or DPM extract with $Fe_2O_3$ as the carrier were studied by Shefner et al. (1982). The DPM component in each of the treatments was administered at concentrations of 1.25, 2.5, or 5.0 mg/week for 15 weeks to groups of 50 male Syrian golden hamsters. The total volume instilled was 3.0 mL (0.2 mL/week for 15 weeks). The DPM and dichloromethane extracts were suspended in physiological saline with gelatin (0.5% w/v), gum arabic (0.5% w/v), and propylene glycol (10% by volume). The $Fe_2O_3$ concentration, when used, was 1.25 mg/0.2 mL of suspension. Controls received vehicle and, where appropriate, carrier particles ($Fe_2O_3$) without the DPM component. Two replicates of the experiments were performed. Adenomatous hyperplasia was reported to be most severe in those animals treated with DPM or DPM plus $Fe_2O_3$ particles and least severe in those animals receiving DPM plus $Fe_2O_3$. Of the two lung adenomas detected microscopically, one was in an animal treated with a high dose of DPM and the other was in an animal receiving a high dose of DPM extract. Although lung damage was increased by instillation of DPM, there was no evidence of tumorigenicity.

### 7.3.3. Mouse Studies

Ichinose et al. (1997a) intratracheally instilled 36 four-week-old male ICR mice per group weekly for 10 weeks with sterile saline or 0.05, 0.1, or 0.2 mg DPM. Particles were collected from a 2.74 L, four-cylinder Isuzu engine run at a steady speed of 1,500 rpm under a load of 10 torque (kg/m). Twenty-four hours after the last instillation, six animals per group were sacrificed for measurement of lung 8-hydroxydeoxyguanosine (8-OHdG). The remaining animals were sacrificed after 12 mo for histopathological analysis. Lung tumor incidence varied from 4/30 (13.3%) for controls to 9/30 (30%), 9/29 (31%), and 7/29 (24.1%) for mice instilled with 0.05, 0.1, and 0.2 mg/week, respectively. The increase in animals with lung tumors compared with controls was statistically significant for the 0.1 mg dose group, the only group analyzed statistically. Increases in 8-OHdG, an indicator of oxidative DNA damage, correlated well with increases in tumor incidences, with the correlation coefficients $r = 0.916$, 0.765, and 0.677 for the 0.05, 0.10, and 0.20 mg DPM groups, respectively.

In a similar study, 33 four-week-old male ICR mice per group were intratracheally instilled weekly for 10 weeks with sterile saline, 0.1 mg DPM, or 0.1 mg DPM from which the organic

constituents were extracted with hexane (Ichinose et al., 1997b). Exhaust was collected from a 2.74 L four-cylinder Izuzu engine run at a steady speed of 2,000 rpm under a load of 6 torque (kg/m). Twenty-four hours after the last instillation, six animals per group were sacrificed for measurement of 8-OHdG. Surviving animals were sacrificed after 12 mo. The incidence of lung tumors increased from 3/27 (11.1%) among controls to 7/27 (25.9%) among those instilled with extracted diesel particles and 9/26 (34.6%) among those instilled with unextracted particles. The increase in number of tumor-bearing animals was statistically significant compared with controls ($p<0.05$) for the group treated with unextracted particles. The increase in 8-OHdG was highly correlated with lung tumor incidence, r = 0.99.

## 7.4. SUBCUTANEOUS AND INTRAPERITONEAL INJECTION STUDIES

### 7.4.1. Mouse Studies

In addition to inhalation studies, Orthoefer et al. (1981) also tested the effects of i.p. injections of DPM on male Strong A mice. Three groups of 30 mice were injected with 0.1 mL of a suspension (particles in distilled water) containing 47, 117, or 235 µg of DPM collected from Fluoropore filters in the inhalation exposure chambers. The exposure system and exposure atmosphere are described in Appendix A. Vehicle controls received injections of particle suspension made up of particulate matter from control exposure filters, positive controls received 20 mg of urethan, and negative controls received no injections. Injections were made three times weekly for 8 weeks, resulting in a total DPM dose of 1.1, 2.8, and 5.6 mg for the low-, medium-, and high-dose groups and 20 mg of urethan for the positive control group. These animals were sacrificed after 26 weeks and examined for lung tumors. For the low-, medium-, and high-dose DPM groups, the tumor incidence was 2/30, 10/30, and 8/30, respectively. The incidence among urethan-treated animals (positive controls) was 100% (29/29), with multiple tumors per animal. The tumor incidence for the DPM-treated animals did not differ significantly from that of vehicle controls (8/30) or negative controls (7/28). The number of tumors per mouse was also unaffected by treatment.

In further studies conducted by Orthoefer et al. (1981), an attempt was made to compare the potency of DPM with that of other environmental pollutants. Male and female Strain A mice were injected i.p. three times weekly for 8 weeks with DPM, DPM extracts, or various environmental mixtures of known carcinogenicity, including cigarette smoke condensate, coke oven emissions, and roofing tar emissions. Injection of urethan or dimethylsulfoxide (DMSO) served as positive or vehicle controls, respectively. In addition to DPM from the Nissan diesel previously described, an 8-cylinder Oldsmobile engine operated at the equivalent of 40 mph was also used to compare emission effects from different makes and models of diesel engine. The mice were sacrificed at 9 mo of age and their lungs examined for histopathological changes. The

only significant findings, other than for positive controls, were small increases in numbers of lung adenomas per mouse in male mice injected with Nissan DPM and in female mice injected with coke oven extract. Furthermore, the increase in the extract-treated mice was significant only in comparison with uninjected controls (not injected ones) and did not occur when the experiment was repeated. Despite the use of a strain of mouse known to be sensitive to tumor induction, the overall findings of this study were negative. The authors provided several possible explanations for these findings, the most likely of which were (1) the carcinogens that were present were very weak or (2) the concentrations of the active components reaching the lungs were insufficient to produce positive results.

Kunitake et al. (1986) conducted studies using DPM extract obtained from a 1983 HD MMC M-6D22P 11-L V-6 engine. Five s.c. injections of DPM extract (500 mg/kg per injection) resulted in a significant ($p<0.01$) increase in subcutaneous tumors for female C57BL mice (5/22 [22.7%] vs. 0/38 among controls). Five s.c. doses of DPM extract of 10, 25, 30, 100, or 200 mg/kg failed to produce a significant increase in tumor incidence. One of 12 female ICR mice (8.3%) and 4 of 12 male ICR mice (33.3%) developed malignant lymphomas following neonatal s.c. administration of 10 mg of DPM extract per mouse. The increase in malignant lymphoma incidence for the male mice was statistically significant at ($p<0.05$) compared with an incidence of 2/14 (14.3%) among controls. Treatment of either sex with 2.5 or 5 mg of DPM extract per mouse did not result in statistically significant increases in tumor incidence.

Additional studies using DPM extract from LD (1.8-L, 4-cylinder) as well as HD engines with female ICR and nude mice (BALB/c/cA/JCL-nu) were also reported (Kunitake et al., 1988). Groups of 30 ICR and nude mice each were given a single s.c. injection of 10 mg HD extract, 10 mg HD + 50 µg 12-O-tetradecanoylphorbol 13-acetate (TPA), 10 mg LD extract + 50 µg TPA, or 50 µg TPA. No malignant tumors or papillomas were observed. One papillomatous lesion was observed in an ICR mouse receiving LD extract + TPA, and acanthosis was observed in one nude mouse receiving only TPA.

In what appears to be an extension of the Kunitake et al. (1986) s.c. injection studies, Takemoto et al. (1988) presented additional data for subcutaneously administered DPM extract from HD and LD diesel engines. In this report, the extracts were administered to 5-week-old and neonatal (<24 h old) C57BL mice of both sexes. DPM extract from HD or LD engines was administered weekly to the 5-week-old mice for 5 weeks at doses of 10, 25, 50, 100, 200, or 500 mg/kg, with group sizes ranging from 15 to 54 animals. After 20 weeks, comparison with a control group indicated a significant increase in the incidence of subcutaneous tumors for the 500 mg/kg HD group (5 of 22 mice [22.7%], $p<0.01$), the 100 mg/kg LD group (6 of 32 [18.8%], $p<0.01$), and the 500 mg/kg LD group (7 of 32 [21.9%], $p<0.01$) in the adult mouse experiments. The tumors were characterized as malignant fibrous histiocytomas. No tumors were observed in

other organs. The neonates were given single doses of 2.5, 5, or 10 mg DPM extract subcutaneously within 24 h of birth. There was a significantly higher incidence of malignant lymphomas in males receiving 10 mg of HD extract and of lung tumors for males given 2.5 mg HD extract and for males given 5 mg and females given 10 mg LD extract. A dose-related trend that was not significant was observed for the incidences of liver tumors for both the HD extract- and LD extract-treated neonatal mice. The incidence of mammary tumors in female mice and multiple-organ tumors in male mice was also greater for some extract-treated mice but was not dose related. The report concluded that LD DPM extract showed greater carcinogenicity than did HD DPM extract.

## 7.5. DERMAL STUDIES

### 7.5.1. Mouse Studies

In one of the earliest studies of diesel emissions, the effects of dermal application of extract from DPM were examined by Kotin et al. (1955). Acetone extracts were prepared from the DPM of a diesel engine (type and size not provided) operated at warm-up mode and under load. These extracts were applied dermally three times weekly to male and female C57BL and strain A mice. Results of these experiments are summarized in Table 7-4. In the initial experiments using 52 (12 male, 40 female) C57BL mice treated with DPM extract from an engine operated in a warm-up mode, two papillomas were detected after 13 mo. Four tumors in 8 surviving of 50 exposed male strain A mice treated with DPM extract from an engine operated under full load were detected 16 mo after the start of treatment. Among female strain A mice treated with DPM extract from an engine operated under full load, 17 tumors were detected in 20 of 25 mice surviving longer than 13 mo. This provided a significantly increased tumor incidence of 85%. Carcinomas as well as papillomas were seen, but the numbers were not reported.

Depass et al. (1982) examined the potential of DPM and dichloromethane extracts of DPM to act as complete carcinogens, carcinogen initiators, or carcinogen promoters. In skin-painting studies, the DPM was obtained from an Oldsmobile 5.7 L diesel engine operated under constant load at 65 km/h. The DPM was collected at a temperature of 100°C. Groups of 40 C3H/HeJ mice were used because of their low spontaneous tumor incidence. For the complete carcinogenesis experiments, DPM was applied as a 5% or 10% suspension in acetone. Dichloromethane extract was applied as 5%, 10%, 25%, or 50% suspensions. Negative controls

**Table 7-4. Tumorigenic effects of dermal application of acetone extracts of diesel particulate matter (DPM)**

| Number of animals | Strain/sex | Sample material | Time to first tumor (mo) | Survivors at time of first tumor | Total tumors | Duration of experiment (mo) |
|---|---|---|---|---|---|---|
| 52 | C57BL/40 F<br>C57BL/12 M | Extract of DPM obtained during warm-up | 13 | 33 | 2 | 22 |
| 50 | Strain A/M | Extract of DPM obtained during full load | 15 | 8 | 4 | 23 |
| 25 | Strain A/F | Extract of DPM obtained during full load | 13 | 20 | 17 | 17 |

Source: Kotin et al. (1955).

received acetone, and positive controls received 0.2% B[a]P. For tumor-promotion experiments, a single application of 1.5% B[a]P was followed by repeated applications of 10% DPM suspension, 50% DPM extract, acetone only (vehicle control), 0.0001% phorbol 12-myristate 13-acetate (PMA) as a positive promoter control, or no treatment (negative control). For the tumor-initiation studies, a single initiating dose of 10% diesel particle suspension, 50% diesel particle extract, acetone, or PMA was followed by repeated applications of 0.0001% PMA. Following 8 mo of treatment, the PMA dose in the initiation and promotion studies was increased to 0.01%. Animals were treated three times per week in the complete carcinogenesis and initiation experiments and five times per week in promotion experiments. All test compounds were applied to a shaved area on the back of the mouse.

In the complete carcinogenesis experiments, one mouse receiving the high-dose (50%) suspension of extract developed a squamous cell carcinoma after 714 days of treatment. Tumor incidence in the B[a]P group was 100%, and no tumors were observed in any of the other groups. For the promotion studies, squamous cell carcinomas with pulmonary metastases were identified in one mouse of the 50% DPM extract group and in one in the 25% extract group. Another mouse in the 25% extract group developed a grossly diagnosed papilloma. Nineteen positive control mice had tumors (11 papillomas, 8 carcinomas). No tumors were observed for any of the other treatment groups. For the initiation studies, three tumors (two papillomas and one carcinoma) were identified in the group receiving DPM suspension and three tumors (two papillomas and one fibrosarcoma) were found in the DPM extract group. These findings were reported to be statistically insignificant using the Breslow and Mantel-Cox tests.

The data from this study indicated that DPM and dichloromethane extracts of these particles are not effective with regard to tumor promotion or initiation. Although these findings were not consistent with those of Kotin et al. (1955) (Table 7-2), the occurrence of a single carcinoma in a strain known to have an extremely low spontaneous tumor incidence may be of importance. Furthermore, a comparison between studies employing different strains of mice with varying spontaneous tumor incidences may result in erroneous assumptions.

Nesnow et al. (1982) studied the formation of dermal papillomas and carcinomas following dermal application of dichloromethane extracts from coke oven emissions, roofing tar, DPM, and gasoline engine exhaust. DPM from five different engines, including a preproduction Nissan 220C, a 5.7-L Oldsmobile, a prototype Volkswagen Turbo Rabbit, a Mercedes 300D, and a HD Caterpillar 3304, was used for various phases of the study. Male and female Sencar mice (40 per group) were used for tumor-initiation, tumor-promotion, and complete carcinogenesis studies. For the tumor-initiation experiments, the DPM extracts were topically applied in single doses of 100, 500, 1,000 or 2,000 µg/mouse. The high dose (10,000 µg/mouse) was applied in five daily doses of 2,000 µg. One week later, 2 µg of the tumor promoter tetradecanoylphorbol

acetate (TPA) was applied topically twice weekly. The tumor-promotion experiments used mice treated with 50.5 µg of B[a]P followed by weekly (twice weekly for high dose) topical applications (at the aforementioned doses) of the extracts. For the complete carcinogenesis experiments, the test extracts were applied weekly (twice weekly for the high doses) for 50 to 52 weeks. Only extracts from the Nissan, Oldsmobile, and Caterpillar engines were used in the complete carcinogenesis experiments.

In the tumor-initiation studies, both B[a]P alone and the Nissan engine DPM extract followed by TPA treatment produced a significant increase in tumor (dermal papillomas) incidence at 7 to 8 weeks postapplication. By 15 weeks, the tumor incidence was greater than 90% for both groups. No significant carcinoma formation was noted for mice in the tumor-initiation experiments following exposure to DPM extracts of the other diesel engines, although the Oldsmobile engine DPM extract at 2.0 mg/mouse did produce a 40 percent papilloma incidence in male mice at 6 mo. This effect, however, was not dose dependent.

B[a]P (50.5 µg/week), coke oven extract (at 1.0, 2.0, or 4.0 mg/week), and the highest dose of roofing tar extract (4.0 mg/week) all tested positive for complete carcinogenesis activity. DPM extracts from only the Nissan, Oldsmobile, and Caterpillar engines were tested for complete carcinogenic potential, and all three proved to be negative using the Sencar mouse assay.

The results of the dermal application experiments by Nesnow et al. (1982) are presented in Table 7-5. The tumor initiation-promotion assay was considered positive if a dose-dependent response was obtained and if at least two doses provided a papilloma-per-mouse value that was three times or greater than that of the background value. Based on these criteria, only emissions from the Nissan were considered positive. Tumor initiation and complete carcinogenesis assays required that at least one dose produce a tumor incidence of at least 20%. None of the DPM samples yielded positive results based on this criterion.

Kunitake et al. (1986, 1988) evaluated the effects of a dichloromethane extract of DPM obtained from a 1983 MMC M-6D22P 11-L V-6 engine. An acetone solution was applied in 10 doses every other day, followed by promotion with 2.5 µg of TPA three times weekly for 25 weeks. Exposure groups received a total dose of 0.5, 5, 15, or 45 mg of extract. Papillomas were reported in 2 of 50 animals examined in the 45 mg exposure group and in 1 of 48 in the 15 mg group compared with 0 of 50 among controls. Differences, however, were not statistically significant.

**Table 7-5. Dermal tumorigenic and carcinogenic effects of various emission extracts**

| | Tumor initiation | | Complete carcinogenesis | Tumor promotion |
|---|---|---|---|---|
| **Sample** | **Papillomas[a]** | **Carcinomas[b]** | **Carcinomas[b]** | **Papillomas[a]** |
| Benzo[*a*]pyrene | +/+[c] | +/+ | +/+ | +/+ |
| Topside coke oven | +/+ | -/+ | ND[d] | ND |
| Coke oven main | +/+ | +/+ | +/+ | +/+ |
| Roofing tar | +/+ | +/+ | +/+ | +/+ |
| Nissan | +/+ | +/+ | -/- | ND |
| Oldsmobile | +/+ | -/- | -/- | ND |
| VW Rabbit | +/+ | -/- | I[e] | ND |
| Mercedes | +/- | -/- | ND | ND |
| Caterpillar | -/- | -/- | -/- | ND |
| Residential furnace | -/- | -/- | ND | ND |
| Mustang | +/+ | -/+ | ND | ND |

[a]Scored at 6 mo.
[b]Cumulative score at 1 year.
[c]Male/female.
[d]ND = Not determined.
[e]I = Incomplete.

Source: Nesnow et al. (1982).

## 7.6. SUMMARY AND CONCLUSIONS OF LABORATORY ANIMAL CARCINOGENICITY STUDIES

As early as 1955, Kotin et al. (1955) provided evidence for tumorigenicity and carcinogenicity of acetone extracts of DPM following dermal application and also provided data suggesting a difference in this potential depending on engine operating mode. Until the early 1980s, no chronic studies assessing inhalation of diesel exhaust, the relevant mode for human exposure, had been reported. Since then, inhalation studies have been emphasized.

Studies using rats and an experimental protocol including long-term exposure at high exposure concentrations (up to 8 mg/m$^3$), resulting in large lung particle loads and a postexposure observation period, were generally positive in demonstrating DPM-induced increases in tumorigenicity. The highest incidences of tumors were reported by Brightwell et al. (1986, 1989). Among female rats exposed for 24 mo and held for their lifetimes, tumors were detected in 24 of 25 animals. This study points out the probable cumulative effects of high exposure concentration (6.6 mg/m$^3$), lengthy daily exposures (16 h/day), exposure in the dark resulting in a probable increase in ventilation and thereby DPM intake, and maintenance of the animals for their lifetimes. In two major studies reported by Heinrich et al. (1986a) and Mauderly et al. (1987), significant but lower lung tumor incidences were observed at the high-dose levels, 15.8% and 12.8%, respectively. Although exposure concentrations differed (7 mg/m$^3$ for Mauderly et al. vs. 4 mg/m$^3$ for Heinrich et al.), the longer daily exposure periods in the Heinrich et al. study, 19 h versus 7 h, would probably result in only slightly differing intakes. More recently, Nikula et al. (1995) provided additional data for rats, and Heinrich et al. (1995) reported on a study involving rats. In the Nikula et al. (1995) report, the percentages of rats (males and females combined) with neoplasms following exposure up to 23 mo were 1% (controls), 6% (2.5 mg/m$^3$ diesel exhaust), and 18% (6.5 mg/m$^3$ diesel exhaust). In the Heinrich et al. (1995) report, the percentages of rats with tumors following exposure up to 23 mo were <1% (controls), 0% (0.8 mg/m$^3$), 6% (2.5 mg/m$^3$), and 22% (7.0 mg/m$^3$).

Ishinishi et al. (1988a, 1988b) reported a 6.5% incidence of lung tumors in rats exposed to a concentration of 4 mg/m$^3$ DPM from a HD engine. In this study, although the concentration was relatively low, duration and length of daily exposure were long (16 h/day for 30 mo). Iwai et al. (1986) reported an increased lung tumor incidence (4/14) in Fischer rats exposed 8 h/day, 7 days/week for 24 mo to a DPM concentration of 4.9 mg/m$^3$. Four of five rats held in clean air for an additional 3 to 6 mo, however, also developed tumors, pointing out again the importance of a long study duration. Iwai et al. (1986) reported the only diesel exhaust inhalation-induced tumor increase at a nonrespiratory site (splenic lymphoma).

Low exposure concentrations and/or short exposure durations were generally used in the negative studies (Karagianes et al. 1981; Lewis et al. 1989; White et al. 1983; Takemoto et al.,

1986). The lowest DPM concentrations resulting in significant positive effects in rats were in the range of 2 to 3 mg/m$^3$.

Inhalation of diesel exhaust induced significant increases in lung tumors in female NMRI mice (Heinrich et al., 1986a; Stöber, 1986) and in female Sencar mice (Pepelko and Peirano, 1983). An apparent increase was also seen in female C57BL mice (Takemoto et al., 1986). In a series of inhalation studies using strain A mice, no increases in lung tumor rates were detected (Orthoefer et al., 1981; Kaplan et al., 1982; Kaplan et al., 1983; White et al., 1983). The only study in which lung tumor incidences were increased in animals exposed to filtered exhaust was reported by Heinrich et al. (1986a) and Stöber (1986) using NMRI mice. In a more recent study by Heinrich et al. (1995) exposure of NMRI and C57BL/6N mice to diesel exhaust (4.5 mg/m$^3$ for up to 23 mo) did not produce a tumorigenic response that was significantly different from that observed in clean air controls.

Attempts to induce significant increases in lung tumors in Syrian hamsters were unsuccessful after inhalation (Heinrich et al., 1982; Heinrich et al., 1986a; Heinrich et al., 1989b; Brightwell et al., 1986) or itr. instillation (Kunitake et al., 1986; Ishinishi et al., 1988b). Neither cats (Pepelko and Peirano, 1983 [see Chapter 4]) nor monkeys (Lewis et al., 1986) developed tumors following 2-year exposure to diesel exhaust. The duration of these exposures, however, may well have been inadequate in these two longer-lived species. Exposure levels were also below the maximum tolerated dose (MTD) in the monkey studies and borderline for detection of lung tumor increases in rats.

Kawabata et al. (1986) demonstrated the induction of lung tumors in Fischer 344 rats following intratracheal instillation of DPM. Grimmer et al. (1987) showed not only that an extract of DPM was carcinogenic when instilled in the lungs of rats but also that most of the carcinogenicity resided in the portion containing PAHs with four to seven rings.

Alternative exposure routes including dermal exposure and s.c. injection in mice provided additional evidence for tumorigenic effects of DPM. Particle extracts applied dermally to mice have been shown to induce significant skin tumor increases in two studies (Kotin et al., 1955; Nesnow et al., 1982). Kunitake et al. (1986) also reported a marginally significant increase in skin papillomas in ICR mice treated with an organic extract from an HD diesel engine. Negative results were reported by Depass et al. (1982) for skin-painting studies using mice and acetone extracts of DPM suspensions. However, in this study the exhaust particles were collected at temperatures of 100°C, a temperature that would minimize the condensation of vapor-phase organics and, therefore, reduce the availability of potentially carcinogenic compounds that might normally be present on diesel exhaust particles. A significant increase in the incidence of sarcomas in female C57Bl mice was reported by Kunitake et al. (1986) following s.c. administration of LD DPM extract at doses of 500 mg/kg. Takemoto et al. (1988) provided

additional data for this study and reported an increased tumor incidence in the mice following injection of LD engine DPM extract at doses of 100 and 500 mg/kg. Results of i.p. injection of DPM or DPM extracts in strain A mice were generally negative (Orthoefer et al., 1981; Pepelko and Peirano, 1983), suggesting that the strain A mouse may not be a good model for testing diesel emissions.

Experiments using tumor initiators such as DEN, B[a]P, DPN, or DBA (Brightwell et al., 1986; Heinrich et al., 1986a; Takemoto et al., 1986) did not provide conclusive results regarding the tumor-promoting potential of either filtered or whole diesel exhaust. A report by Heinrich et al. (1982), however, indicated that filtered exhaust may promote the tumor-initiating effects of DEN in hamsters.

Several reports (Wong et al., 1986; Bond et al., 1990) affirm observations of the potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. These findings are discussed in more detail in Chapter 9. Evidence for the mutagenicity of organic agents present in diesel engine emissions is also provided in Chapter 8.

It appears reasonably certain that with adequate exposures, inhalation of diesel exhaust will induce lung cancer in rats. The relationship between exposure levels and response, however, is less clearcut. Although significant increases in lung tumors were not reported at concentrations less than about 2 $mg/m^3$, the response at higher concentrations varies considerably. A significant percentage of this variation can probably be attributed to the exposure regime. A better method than concentration alone for assessing exposure-response relationships could be achieved by comparing cumulative exposure (concentration × daily exposure duration × days of exposure). Only those studies conducted for a sufficient length of time ($\geq$24 mo) for expression of carcinogenic responses have been included in this analysis. Examination of the rat data (shown in Table 7-6 and plotted in Figure 7-1) reveals that most studies indicate a trend of increasing tumor incidence at exposures exceeding $1 \times 10^4$ $mg \cdot hr/m^3$.

A similar comparison could not be adequately made for mice, because experimental designs were not comparable. The study reported by Stöber (1986), for example, involved lifetime exposure following weaning. In the studies reported by Pepelko and Peirano (1986), however, Sencar mice were exposed from conception, when they are presumably more sensitive to tumor induction, and sacrificed at 15 mo, whereas strain A mice were sacrificed at 9 mo of age because of the rapid increase in the incidence of lung tumors in controls. The successful

**Table 7-6. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust**

| Study | Exposure rate/duration (h/week, mo) | Total exposure time (h) | Particle concentration ($mg/m^3$) | Cumulative exposure ($mg \cdot h/m^3$) | | Tumor incidence (%)[a] |
|---|---|---|---|---|---|---|
| | | | | Per week | Total | |
| Mauderly et al. | 35, 30 | 4,200 | 0 | 0 | 0 | 0.9 |
| (1987) | 35, 30 | 4,200 | 0.35 | 12.25 | 1,470 | 1.3 |
| | 35, 30 | 4,200 | 3.5 | 122.5 | 14,700 | 3.6 |
| | 35, 30 | 4,200 | 7.1 | 248.5 | 29,820 | 12.8 |
| Nikula et al. (1995) | 80, 23 | 7,360 | 0 | 0 | 0 | 1.0 |
| | 80, 23 | 7,360 | 2.5 | 200.0 | 18,400 | 7.0 |
| | 80, 23 | 7,360 | 6.5 | 520.0 | 47,840 | 18.0 |
| Heinrich et al. | 95, 35 | 13,300 | 0 | 0 | 0 | 0 |
| (1986b) | 95, 35 | 13,300 | 4.24 | 402.8 | 56,392 | 17.8 |
| Heinrich et al. (1995) | 90, 24 | 8,640 | 0 | 0 | 0 | 0 |
| | 90, 24 | 8,640 | 0.8 | 72.0 | 7,400[b] | 0 |
| | 90, 24 | 8,640 | 2.5 | 225.0 | 21,800[b] | 5.5 |
| | 90, 24 | 8,640 | 7.0 | 630.0 | 61,700[b] | 22.0 |
| Ishinishi et al. | 96, 30 | 11,520 | 0 | 0 | 0 | 3.3 |
| (1988a) | 96, 30 | 11,520 | 0.1 | 9.6 | 1,152 | 2.4 |
| (Light-duty engine) | 96, 30 | 11,520 | 0.4 | 38.4 | 4,608 | 0.8 |
| | 96, 30 | 11,520 | 1.1 | 105.6 | 12,672 | 4.1 |
| | 96, 30 | 11,520 | 2.3 | 220.8 | 26,496 | 2.4 |
| (Heavy-duty engine) | 96, 30 | 11,520 | 0 | 0 | 0 | 0.8 |
| | 96, 30 | 11,520 | 0.5 | 48.0 | 5,760 | 0.8 |
| | 96, 30 | 11,520 | 1.0 | 96.0 | 11,520 | 0 |
| | 96, 30 | 11,520 | 1.8 | 172.8 | 20,736 | 3.3 |
| | 96, 30 | 11,520 | 3.7 | 355.2 | 42,624 | 6.5 |

**Table 7-6. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust (continued)**

| Study | Exposure rate/duration (h/week, mo) | Total exposure time (h) | Particle concentration ($mg/m^3$) | Cumulative exposure ($mg \cdot h/m^3$) | | Tumor incidence (%)[a] |
|---|---|---|---|---|---|---|
| | | | | Per week | Total | |
| Brightwell et al. | 80, 24 | 7,680 | 0 | 0 | 0 | 1.2 |
| (1989) | 80, 24 | 7,680 | 0.7 | 56.0 | 5,376 | 0.7 |
| | 80, 24 | 7,680 | 2.2 | 176.0 | 16,896 | 9.7 |
| | 80, 24 | 7,680 | 6.6 | 528.0 | 50,688 | 38.5 |
| Kaplan et al. (1983) | 140, 15 | 8,400 | 0 | 0 | 0 | 0 |
| | 140, 15 | 8,400 | 0.25 | 35.0 | 2,100 | 3.3 |
| | 140, 15 | 8,400 | 0.75 | 105.0 | 6,300 | 10.0 |
| | 140, 15 | 8,400 | 1.5 | 210.0 | 12,600 | 3.3 |
| Iwai et al. (1986) | 56, 24 | 5,376 | 0 | 0 | 0 | 0 |
| | 56, 24 | 5,376 | 4.9 | 274.4 | 26,342 | 36.8 |
| Takemoto et al. | 16, 18-24 | 1,152-1,536 | 0 | 0 | 0 | 0 |
| (1986) | 16, 18-24 | 1,152-1,536 | 2-4 | 32-64 | 3,456-4,608 | 0 |
| Karagianes et al. | 30, 20 | 2,400 | 0 | 0 | 0 | 0 |
| (1981) | 30, 20 | 2,400 | 8.3 | 249 | 19,920 | 16.6 |

[a]Combined data for males and females.
[b]As reported in Heinrich et al. (1995).

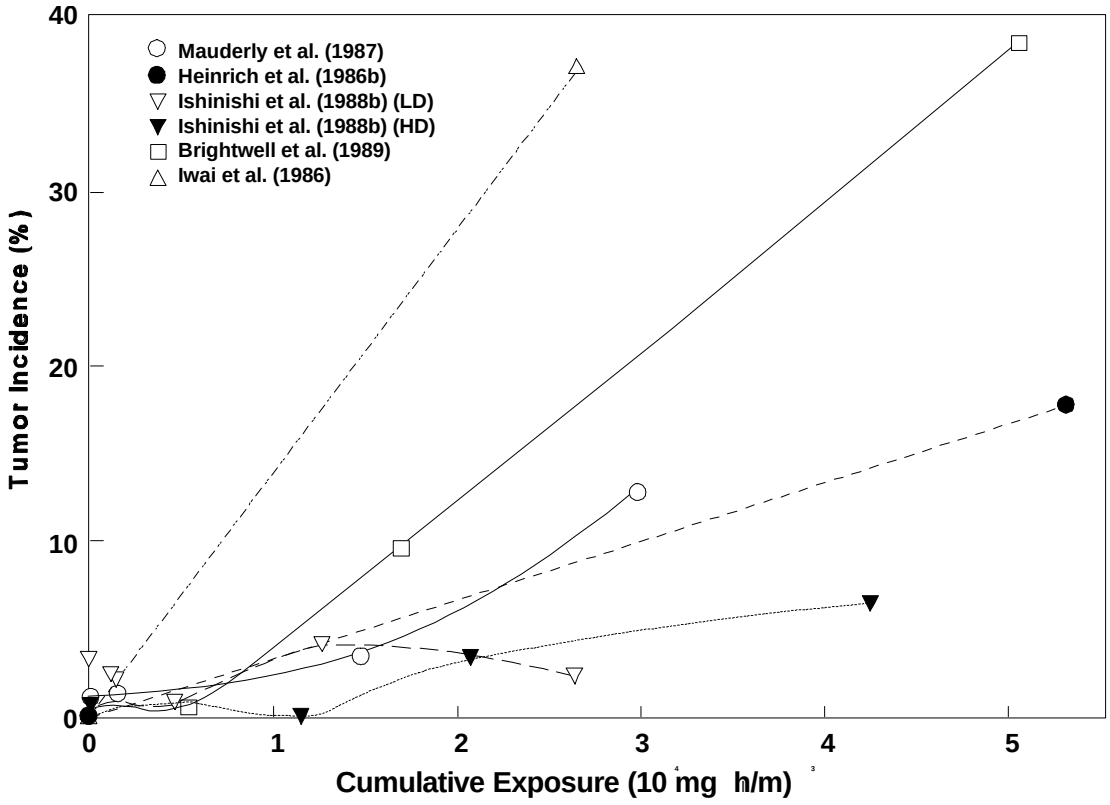


**Figure 7-1. Cumulative exposure data for rats exposed to whole diesel exhaust.**

induction of lung tumors in mice by Ichinose et al. (1997a,b) via intratracheal instillation may be the result of focal deposition of larger doses.

Although the preceding analysis accounts for total inspired dose, it does not account for delayed particle clearance at higher exposure levels. The analysis might be improved further by comparing tumor response with lung burden of particulate matter. This analysis could account for not only differences in exposure times but also for overload inhibition of clearance at high exposure levels. Although such data were not available for all studies, it could be estimated by using available estimates of respiration along with deposition and clearance models. The extrapolation models (Appendix A) and the qualitative/quantitative evaluations of Chapter 11 attempt this relative to human exposure.

To evaluate accurately the carcinogenic risk to humans from diesel engine emissions, it is important to ascertain the fraction or fractions of exhaust responsible for inducing lung tumors. Several of the previously discussed studies indicated that only whole (unfiltered) diesel exhaust is tumorigenic or carcinogenic and that these responses are eliminated or greatly minimized in exposures to filtered diesel exhaust. Data for NMRI and C57BL/6N mice exposed to whole diesel exhaust (4.5 $m/m^3$), filtered exhaust, or clean air for up to 23 mo showed no significant increases in tumor incidences for mice exposed to filtered exhaust relative to clean air controls

(Heinrich et al., 1995). In one study (Stöber, 1986), however, a significant increase in lung tumors was seen in mice exposed to filtered exhaust. Heinrich et al. (1982) also provided some evidence suggesting that the gaseous fraction promoted the tumorigenic effects of DEN. Nevertheless, because of the lack of positive data in rats and the limited positive data in mice, the tumorigenicity of the gaseous fraction must be considered to be unresolved.

The importance of DPM has been affirmed for the tumorigenic response observed in rats (Heinrich et al., 1995; Nikula et al., 1995). Evidence for the importance of the carbon core was initially provided by studies of Kawabata et al. (1986), which showed induction of lung tumors following intratracheal instillation of carbon black that contained no more than traces of organics and studies of Heinrich (1990) that indicated that exposure via inhalation to carbon black (Printex 90) particles induced lung tumors at concentrations similar to those effective in DPM studies. Induction of lung tumor by other particles of low solubility such as titanium dioxide (Lee et al., 1986) confirmed the capability of particles in inducing lung tumors. Pyrolyzed pitch, on the other hand, essentially lacking a carbon core but having much higher PAH concentrations than DPM, also was effective in tumor induction (Heinrich et al., 1986b; 1994).

The relative importance of the adsorbed organics, however, remains to be elucidated and is of some concern because of the known carcinogenic capacity of some of these chemicals. These include polycyclic aromatics as well as nitroaromatics as described in Chapter 2. Organic extracts of particles also have been shown to induce tumors in a variety of injection, intratracheal instillation, and skin-painting studies, and Grimmer et al. (1987) have, in fact, shown that the great majority of the carcinogenic potential following instillation resided in the fraction containing four- to seven-ring PAHs.

In summary, based on positive inhalation exposure data in rats and mice, intratracheal instillation in rats, and injection or skin painting in mice and supported by positive mutagenicity studies, the evidence for carcinogenicity of diesel exhaust is considered to be adequate. The contribution of the various fractions of diesel exhaust to the carcinogenic response is less certain. The effects of the gaseous phase are equivocal. The presence of known carcinogens adsorbed to diesel particles and the demonstrated tumorigenicity of particle extracts in a variety of injection, instillation, and skin-painting studies suggest carcinogenic potential for the organic fraction. Studies showing that insoluble particles (e.g., carbon black, $TiO_2$) can also induce tumors, on the other hand, have provided definitive evidence that the carbon core of the diesel particle is instrumental in the carcinogenic response observed in rats.

A summary of studies assessing the tumorigenic and carcinogenic effects in laboratory animals following inhalation exposure to diesel exhaust is presented in Table 7-1.

## 7.7. REFERENCES


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